

CRADA FINAL REPORT

Distributed Physical and Molecular Separations for Selective Harvest of Higher Value Wheat Straw Components Project

Idaho National Laboratory

and

**Idaho Department of Water Resources
Energy Division, Idaho Wheat
Commission, and Inland Northwest
Research Alliance, Inc.**

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Selective Harvest of Higher Value Wheat Straw Components

Recipient: State of Idaho, Idaho Department of Water Resources, Boise, ID 83720

WBS#: 1.1.2 CID#: GO10614

Reporting Period: October 2000 to September 2004

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Executive Summary

Purpose and Scope

Wheat straw (*Triticum aestivum* L.) is an abundant source of plant fiber. It is regenerated, in large quantities, every year. At present, this potentially valuable resource is greatly under-exploited. Most of the excess straw biomass (i.e., tonnage above that required for agronomic cropping system sustainability) is managed through expensive chopping/tillage operations and/or burnt in the field following harvest, resulting in air pollution and associated health problems. Potential applications for wheat straw investigated within this project include energy and composites manufacture. Other methods of straw utilization that will potentially benefit from the findings of this research project include housing and building, pulp and paper, thermal insulation, fuels, and chemicals.

This project focused on components of the feedstock assembly system for supplying a higher value small grains straw residue for 1) gasification/combustion and 2) straw-thermoplastic composites. This project was an integrated effort to solve the technological, infrastructural, and economic challenges associated with using straw residue for these bioenergy and bioproducts applications.

The objective of the research is to contribute to the development of a low-capital distributed harvesting and engineered storage system for upgrading wheat straw to more desirable feedstocks for combustion and for straw-plastic composites. We investigated two processes for upgrading wheat straw to a more desirable feedstock:

1. An efficient combine-based threshing system for separating the internodal stems from the leaves, sheaths, nodes, and chaff.
2. An inexpensive biological process using white-rot fungi to improve the composition of the mechanically processed straw stems.

Key Results

Physical Fractionation of Straw for Gasification/Combustion

Mechanical threshing and separation processes achieved about a 75% pure cereal straw stem fraction, and reduced the cereal straw stem fraction total ash content by 23% and the silica component of the ash by 44%. However, while mechanical separation reduced total straw ash, it was less effective at reducing the alkali content of the ash. Field washing the straw by irrigation had the greatest effect in reducing straw ash alkali content. As little as 13 mm of water applied to straw windrows resulted in a 54% reduction in straw ash potassium and a 55% reduction in straw ash sodium, which are well-known low-temperature eutectic alkali metals. The overall effect of field washing was a 0.94 kg•Mbtu⁻¹ alkali reduction, which increased the overall fusion temperature of the ash more than 93°C in an oxidizing atmosphere and more than 149°C in a reducing atmosphere. Grinding the straw to less than 13 mm allowed pneumatic conveyance of the material into the conversion systems and achieved packing densities of greater than 160 kg/m³ for transportation.



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Fungal Pretreatment of Straw in Engineered Storage Systems for Thermoplastic Composites

Laboratory culturing studies showed that by limiting nitrogen and providing sufficient inoculum, it was possible to operate a selective fungal degradation system without prior sterilization of the wheat straw. Using these conditions, unsterile wheat straw stems, with *Pleurotus ostreatus* cultured on straw at constant temperature, produced selective degradation rates and conversions (i.e., depolymerized lignin, consumed hemicellulose, and minimized cellulose removal) that increased with both moisture and inoculum. A regression analysis indicated that system performance was quite stable with respect to inoculum and moisture content after 6 weeks of treatment in laboratory straw pretreatment culture systems.

The mechanical properties of straw-plastic composites produced with untreated straw were comparable to those of wood-plastic composites. Straw-plastic composites incorporating straw degraded with non-dominant *P. ostreatus* cultures did not have a statistically significant (α -value of 0.05) influence on either modulus of rupture or modulus of elasticity of straw-plastic composites. However, the mechanical properties of straw-plastic composites produced with untreated straw were comparable to those of wood-plastic composites based on pine flour. In straw-plastic composites with 55% untreated straw, water sorption and thickness swell were higher than for wood-plastic composites based on pine filler. However, those properties were improved in the straw-based composite when 75% untreated straw was used. Water sorption and thickness swell of straw-plastic composites were inferior to wood-plastic composites at filler levels below 55%. Straw-plastic composites using non-dominant fungal (nonselectively) degraded wheat straw demonstrated less resistance to water sorption and thickness swell than straw-plastic composites using untreated straw. This may primarily reflect the fungal degradation of hydrophobic cell wall components (lignin and hemicelluloses) in treated straw, resulting in a relatively more hydrophilic substrate compared to untreated straw. Between 100 and 300°C, fungal degraded straw appeared thermally less stable than non-inoculated straw but this did not have any apparent effect on the extrusion process.

Conclusions

Physical Fractionation of Straw for Gasification/Combustion

Based on the results of this study, any of the processed wheat straw samples could successfully be burned in a fluidized bed in either a combustion or staged combustion process. Washing and separation pretreatments were effective in removing substantial amounts of alkali metals, chlorine, sulfur, calcium oxide, and silica, which improved the combustion process. More importantly, the type or combination of pretreatments applied can selectively and predictably alter biomass constituents to benefit an intended bioenergy conversion end use. From a practical perspective, field washing or selective harvest of straw fractions can be done with existing farm machinery and could fit current farm enterprise practices.

Fungal Pretreatment of Straw in Engineered Storage Systems for Thermoplastic Composites

Scale-up of fungal pretreatment to a representative engineered storage and pretreatment system presented many challenges with respect to establishing dominant selectively degrading *P. ostreatus* fungal cultures. At the laboratory-scale, straw *P. ostreatus* inoculum source/inoculation methods or straw sterilization both proved effective at establishing stable *P. ostreatus* cultures that dominated indigenous species. However, in the less controlled



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environment of the larger straw pretreatment piles, establishing truly dominant *P. ostreatus* cultures could not be fully realized using the inoculation methods employed. Therefore, larger-scale straw degradation tests did not produce the level of selective fungal degradation that was demonstrated in the laboratory.

The results obtained in the present study indicate that wheat straw is a promising alternative to wood fillers in the production of extruded thermoplastic composites, pending an improvement in the hydrophobicity of straw-plastic composite at commercially relevant filler levels. As an alternative to wood fillers, untreated straw produced a superior straw composite plastic to straw degraded with non-dominant *P. ostreatus* fungal cultures. Challenges with establishing dominant *P. ostreatus* fungal cultures in larger-scale systems, such as inoculation method, will need to be solved before in-storage preprocessing can become a viable way to enhance to the overall process.

Recommendations

This research clearly demonstrated the benefits and challenges associated with value-added processing steps as part of the feedstock assembly system. These feedstock assembly processing operations must be considered and tested with respect to the intended end uses, and the logistics and costs of the whole system. The fundamental point to emphasize is that biomass feedstock assembly processes can have a dramatic positive or negative impact on downstream biorefining processes. For example, combine-based fractionation alone very selectively lowered silica mineral levels of the straw stems, but due to the high selectivity of the minerals that were altered, the potassium-silica ratio of these fractionated stems actually became unfavorable for direct gasification without additional treatments (e.g., water wash). However, in a paper mill or some other process application that would have washed the potassium away as a natural part of the process, a highly selective silica reduction should prove most beneficial. As such, from this study and others, there is good evidence that physically-based fractionation of biomass has the potential to increase the value and consistency of biomass for biorefining applications, but fractionation technologies must be developed as an integral part of the intended biorefining process.

Challenges with the complicated biology of an engineered storage system that involves biological preprocessing of the biomass prior to biorefining are no less daunting. In the controlled environment of the laboratory, biological processes are relatively easy to control for achieving an intended preprocessing objective. However, implementing those same processes in the uncontrolled environment of a pile is technically and logistically more challenging. Much more research on the post-harvest physiology and microbial ecology of biomass storage systems is needed for developing low-cost (even passive) biomass pretreatment/preprocessing systems. In the laboratory, the value of such biological preprocessing has been clearly demonstrated, but how to implement such value-added technologies into low-cost and simple to operate biomass piles remains a significant R&D barrier.



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Project Summary

Introduction

Wheat straw (*Triticum aestivum* L.) is an abundant source of plant fiber. It is regenerated, in large quantities, every year. The total worldwide production of cereal straw is estimated to exceed 2900 million tons each year (Sun et al. 2004). At present, this potentially valuable resource is greatly under-exploited. Most of the straw biomass is burnt in the field following harvest, resulting in air pollution and associated health problems. Potential applications for wheat straw encompass the housing and building composites manufacture (Sauter 1996, Zhang et al. 2003, Boquillon et al. 2004), thermal insulation (Strivastava and Gupta 1990), and energy (Kaltschmitt et al. 1997) sectors of our economy.

This project focused on components of the feedstock assembly system for supplying a higher value small grains straw residue for 1) gasification/combustion and 2) straw-thermoplastic composites. This project was an integrated effort to solve the technological, infrastructural, and economic challenges associated with using straw residue for these bioenergy and bioproducts applications, as outlined in Figure 1.

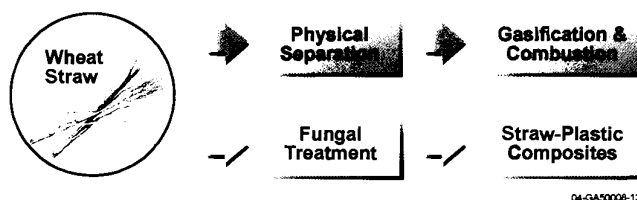


Figure 1. Wheat straw stems were separated from the leaves and nodes, then tested for gasification and combustion or pretreated and tested for use in thermoplastic composites.

The objective of the research was to contribute to the development of a low-capital distributed harvesting and engineered storage system for upgrading wheat straw to more desirable feedstocks for combustion and for straw-thermoplastic composites. We investigated two processes for upgrading wheat straw to a more desirable feedstock:

1. An efficient combine-based threshing system for separating the internodal stems from the leaves, sheaths, nodes, and chaff.
2. An inexpensive biological process using white-rot fungi to improve the composition of the mechanically-processed straw stems.

This report first presents the results of the research on physical separation of undesirable straw components and gasification and combustion tests and then the results on fungal preprocessing and straw-thermoplastics production and testing. A list of publications presenting the results of this work is given in Appendix A.

Results – Straw Residue Pretreatment/Fractionation for Gasification and Combustion Bioenergy Conversion Processes

Recovery of chemicals, energy, and fibers from the large quantity of wheat straw produced annually in the U.S. offers attractive economic potential. However, cost effective straw utilization must be preceded by removal of undesirable components such as silica and alkali minerals. These materials reside primarily in the leaves, sheath nodes, and waxy cuticle layers. Thus, physical separation of these undesirable plant parts should yield straw stem segments in



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physical and chemical form appropriate for fuel, chemical, thermoplastic composite, and pulp fiber production.

Task 1a – Physical Separation of Straw Residue, Laboratory Optimization of Straw Fractionation

Investigators: William T. McKean and Mark Lewis

Performing Institution: University of Washington

Wood chip refiners used in the pulp and paper industry have been adapted to process agricultural residues. Laboratory work was done at the University of Washington with a 12 in. diameter refiner and associated equipment to determine conditions for optimum physical processing to produce clean internodal straw stems.

The schematic of material flows and process steps is shown in Figure 2. A warm water soak softens plant parts and promotes fiberization in the refiner. The water soak also dissolves a portion of the water soluble material present in the plant.

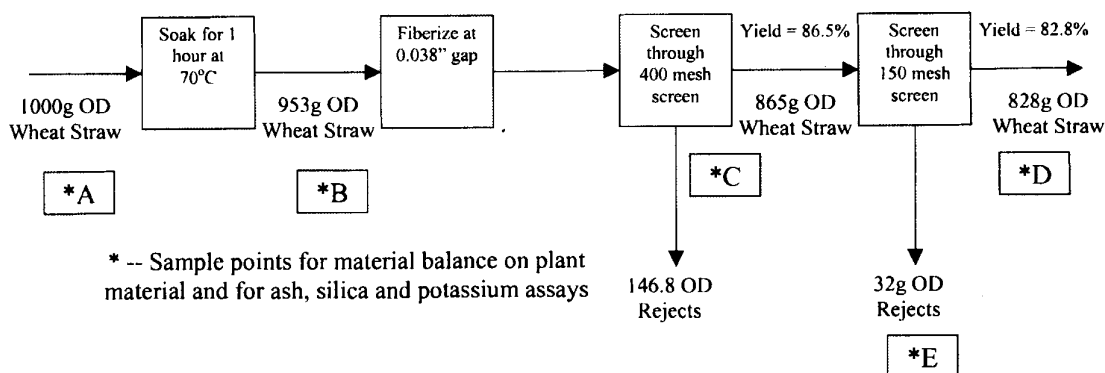


Figure 2. Process sequence for preprocessing all wheat straw cultivars. Straw balance yields cited in this figure are for the Boundary cultivar (see Table 1).

The refining step breaks leaves, sheath, and nodes into small particles and fiberizes internodal stem segments. Much of the fine particles originating from epidermal layers, leaves, sheath, and nodes are rejected from the process in the two screening steps.

Each wheat cultivar has a different physical and chemical composition that results in different material balances in this process sequence. The straw material balance values shown in Figure 2 represent the weight loss for the Boundary cultivar processed in this sequence. The process conditions shown in Figure 2 come from optimization studies completed in winter/spring 2001. Notice that approximately 18% of the plant material passes through the screens and is rejected under these conditions. In addition, the reject streams contain a substantial portion of ash, silica, and potassium originally present in the plant material. Detailed values for these substances and for each cultivar tested in this project are given in the following section.



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Warm Water Soaking

Water soaking of straw serves three functions in this process sequence. First, softening predisposes the leaves, sheath, and nodes to break into fine particle size during fiberization in the refiner. As a result these undesirable parts of the plant are rejected in the later screening steps.

Second, soaking promotes fiberization of internodal stem segments into "slivers" with very little fiber breakage. The short "slivers" will readily pass through process equipment such as combustion unit feeders, extractor units, and feed systems for various pressurized chemical reactors.

Third, soaking will dissolve a portion of the inorganic ash material, potassium salts, and organic plant substances (such as waxes and hemicelluloses). Removal of these materials will improve combustion performance, extraction/chemical reactions, chemical production, performance of thermoplastic composites, and pulp production.

Wheat straw mass balances are shown in Table 1 for each of the wheat cultivars included in this study. Notice that water soaking removes from 1 to 8% of the plant weight. The range in dissolved material likely depends on the chemical composition of each cultivar and on the stem thickness, density, and structure. For example, differences in partially water-soluble inorganic components such as potassium salts and in organic components such as extractives, hemicellulose, and lignin could contribute to the differences between cultivars.

Table 1. Wheat straw mass balance for preprocessing sequences.

Sample	Boundary	Whitebird	Westbred	Stevens
Unprocessed	100.0%	100.0%	100.0%	100.0%
Soaked	95.3%	92.0%	94.9%	99.2%
Refined + 400 Mesh Screen	90.2%	91.3%	95.0%	97.3%
150 Mesh Screen Accepts	86.5%	74.1%	87.2%	85.4%
150 Mesh Screen Rejects	3.7%	17.2%	6.3%	11.9%
Overall Yield of Pulp (based on unprocessed oven dry straw)	82.8%	56.9%	80.9%	73.5%

The sequence of soaking, refining, and screening results in weight loss for two reasons. During each of these three operations, plant material partially dissolves in water. For example, soluble inorganic salts and low molecular weight organic materials such as wax, hemicellulose, and some lignin dissolve in water in all three stages.

Second, some of the plant material broken into small particles during fiberization will pass through 400 and 150 mesh screens. Rejecting these small suspended solid particles contributes to loss in fiber product weight.

We can interpret the reasons for weight loss by comparing results in Tables 1, 2, and 3. Using the Boundary cultivar in Table 1 as an example, during the soaking stage, 4.7% of the oven dry, unprocessed straw weight was lost. Notice in Table 2 that unprocessed Boundary contained 8.6% ash and that soaking reduced the ash to 5.4%. Consequently, about 1/3 of the total weight loss $((4.7 - (8.6 - 5.4)) / 4.7)$ was due to dissolution of the inorganic material measured as ash. The remainder of weight loss must have been a mixture of partially soluble organic waxes, hemicellulose, lignin, and other dissolved or suspended plant material.



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Table 2. Wheat straw ash balance.

Sample	Boundary	Whitebird	Westbred	Stevens
Unprocessed	8.6%	9.9%	9.5%	8.5%
Soaked	5.4%	4.6%	5.9%	8.1%
Refined + 400 Mesh Screen	4.2%	5.2%	6.3%	6.5%
150 Mesh Screen Accepts	4.0%	3.5%	3.1%	5.3%
Overall Ash Rejection	53.5%	69.6%	67.9%	37.6%

Potassium balances during these process steps are particularly important since this alkali earth metal is detrimental to several downstream process steps in combustion, chemical recovery, fiber-thermoplastic composites, and fiber production. Data in Table 3 show the fate of this metal species. During soaking about 80% of the original potassium dissolves in the water. Water soaking conditions (time, temperature, and pH) will have a strong affect on potassium removal. However, these results and field sprinkler studies (see field-scale fractionation studies below) show that substantial potassium removal will occur under mild exposure to water.

Table 3. Wheat straw potassium and silica balance.

Sequential Straw Sample Treatment	Boundary		Whitebird		Westbred		Stevens	
	<i>K and Si % of total plant straw residue mass</i>							
	K	Si	K	Si	K	Si	K	Si
Unprocessed	1.23	0.08	1.38	0.09	1.27	0.09	1.18	0.07
Soaked	0.23	0.07	0.25	0.08	0.28	0.08	0.20	0.06
Fiberized	0.01	0.06	0.01	0.07	0.01	0.08	0.01	0.05
	<i>% of original K and Si removed as a result of all treatments</i>							
Total K and Si removed	99%	25%	99%	22%	99%	11%	99%	29%

Refining and 400 Mesh Screening

Fiberization of the straw during refining exposes more of the soluble plant material to water. As a result, refining followed by 400 mesh screening contributes additional weight loss of 1–5% (Table 1), depending on the cultivar. Comparison with Table 2 shows that from 0 to 75% of that weight loss comes from ash removal. For example, in the case of Boundary cultivar 23.5% $((5.4-4.2)/5.4)$ of the weight loss occurs by ash removal. The Whitebird and Westbred experience no ash loss within experimental error, while over 75% of the weight loss from Stevens cultivar originates from ash loss. The remainder of the loss comes from small, suspended particles that pass through the screen.

Clearly, different cultivars will behave differently in processing steps depending on plant chemistry and morphology.

Results in Table 3 show that significant potassium removal occurs during this process step. For example, with the Boundary cultivar the potassium level drops from 0.23% to 0.01%. Thus, 18.3% $((0.23-0.01)/(5.4-4.2))$ of the weight loss in this process step is caused by potassium dissolution.



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150 Mesh Screening

This larger screen retains the useful pulp fibers and allows further rejection of small, ash-rich and potassium-rich particulate materials. Figure 3 contains the fiberized straw weight yield and the straw ash content for each of the process steps.

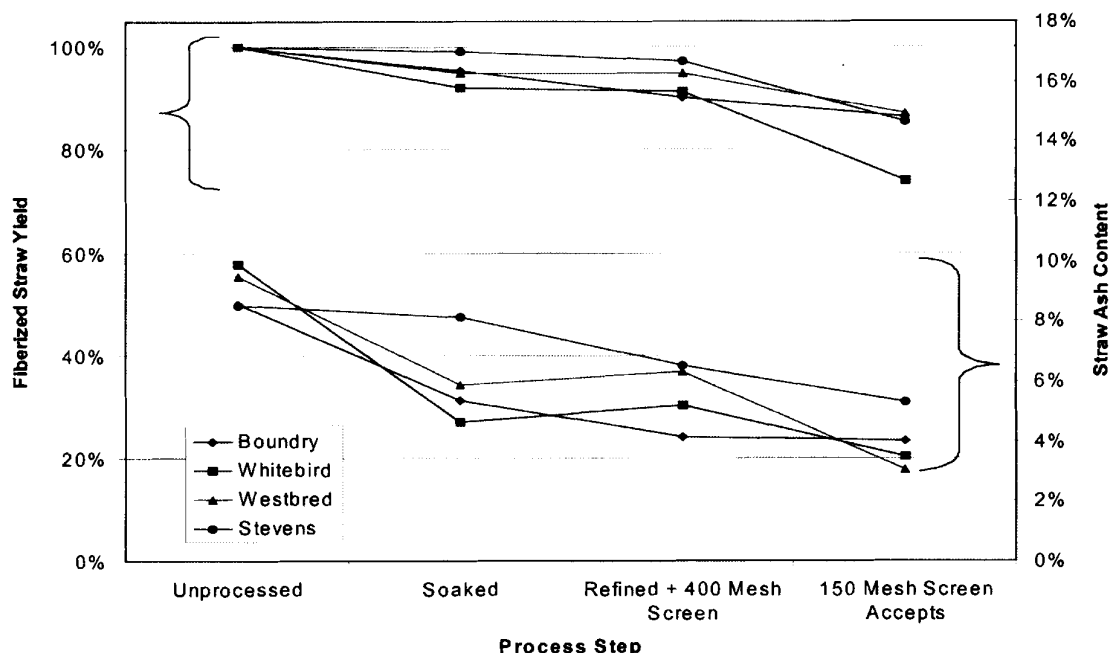


Figure 3. Straw and ash balance for each processing step.

Silica Balance

Silica is much less water soluble than potassium. Depending on the cultivar, from 10 to 30% of the total plant silica was removed (Table 3) in this process sequence. About equal amounts of silica are removed from the straw in the soaking and the fiberization and screening steps. The total silica removal was similar to the total weight loss, suggesting that silica is uniformly distributed within the plant and strongly adheres to plant morphological structures. As a result, the silica removal only occurs when the plant part to which it is attached is removed.

Task 1b – Physical Separation of Straw Residue, Field-Scale Straw Fractionation

Investigators: J. Richard Hess,¹ Reed L. Hoskinson,¹ David N. Thompson,¹ Duane R. Grant,² Judi Steciak³

Performing Institutions: ¹Idaho National Engineering and Environmental Laboratory, ²Idaho Wheat Commission/Grant 4-D Farms, ³University of Idaho

These studies were conducted to evaluate compositional and format changes in cereal straw residues resulting from field-scale harvester fractionation, field washing, and grinding, as well as to assess the benefit of these pretreatments to downstream conversion processes. The effect of these pretreatments will be more fully described in the feedstock testing sections below.



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As demonstrated by the University of Washington studies, pretreatment of biomass components removes or alters undesirable components of the biomass, which is necessary to optimally use biomass in bioenergy conversion. By incorporating selective harvest and pretreatment process steps into the biomass feedstock supply chain, undesirable parts of the straw residue can be separated out and left in the field to maintain soil health, or directed to profitable end uses (e.g., livestock markets). Additionally, these pretreatment steps add value to the cereal straw biomass downstream processing steps, thus making the overall bioenergy conversion process more environmentally sustainable and economically competitive.

These field-scale straw fractionation studies used wheat straw variety Westbred 936, hard red spring, grown in 2001. The wheat crop was produced in Rupert, Idaho, USA, under a high input production system with center pivot irrigation. The grain was harvested using a conventional 9600 John Deere combine with a 7.3 m (24 ft) header. With the straw chopper disengaged, the wheat straw was dropped into a windrow directly behind the combine.

Pretreatments of 13, 25, 50, and 76 mm of water were applied to straw windrows by rain and the center pivot irrigation system to remove soluble mineral components of wheat straw. This washing step was a similar treatment to the water soaking the straw in the University of Washington studies. The first 13 mm was from rainwater, and the remaining water was applied with the irrigation system. Unwatered straw windrows (0 mm of water, baseline) were collected into 1.2 x 1.2 x 2.4 m bales prior to watering, and water-treated windrows were baled after field drying to less than 10% moisture.

Washed and unwashed straw bales were shredded and reprocessed through a stationary 9600 John Deere conventional grain combine to separate the chaff and produce a stem-enriched straw product. The chaff and stem streams were kept separate by conveying the combine chaffer discharge into one truck and the straw walker discharge to another. The stem-enriched fraction was kept for further testing, and the chaff fraction was discarded. Each reprocessed bale was sampled before and after separation to monitor the chemical, mineral, and anatomical separation effect of the combine re-threshing process.

The washing and combine re-threshing pretreatment processes produced four lots of straw material for multi-day fluidized-bed pilot-scale combustion and staged combustion testing. These lots were: 1) Unwashed and Unseparated (UU) or untreated straw, 2) Washed Unseparated (WU), 3) Unwashed Separated (UW), and 4) Washed Separated (WS). The WS straw lot was ground and formed into 0.64 cm diameter pellets. The UU, US, and WU lots were processed to a shredded 1.27 cm minus sized material.

Anatomical composition of the straw was determined by manually sorting straw samples into categories of chaff (leaf, sheath, and stem pieces; awns; racas; and fines), sheath still attached to stem, stems, and nodes. The weight percent of each fraction was then determined.

Straw compositions (carbohydrates and minerals) were measured using the quantitative saccharification technique and muffle furnace ashing, as previously described (Hess et al. 2003). Straw ash composition and characteristic analyses were conducted by Hazen Laboratories, Golden, Colorado, USA, following ASTM procedures (ASTM-D1857, 2003).



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Pretreatment Effect on Straw Properties

The two field-scale pretreatment processes altered the composition of wheat straw in a manner predictably similar to that observed with the laboratory fractionation studies. Both the washing and separation treatments reduced straw ash content (Table 4). The washing treatment alone was most effective at reducing the alkali content of wheat straw. Potassium and chlorine are known to readily move through the plant from older to younger leaves and then to storage organs (Salisbury and Ross 1992). These elements are highly water-soluble, and could be easily removed with a water wash

(Jenkins et al. 1996). Applying of 25 mm of water removed 56% of the potassium and 75% of the chlorine (Fig. 4).

Cumulative water applications up to 76 mm did not remove additional amounts of potassium or chlorine, though it has been reported that application of significantly more water (100 mm) could achieve an 80% or greater reduction in these mineral levels (Sander 1997). These results, suggesting that the lowest amount of applied water is sufficient to remove most of these minerals, puts forward the possibility that straw washing could be incorporated with fall irrigation practices for tillage and fall crop planting. This would expand the geographic areas where such a washing practice could be implemented from areas of high winter rainfall to arid irrigated areas.

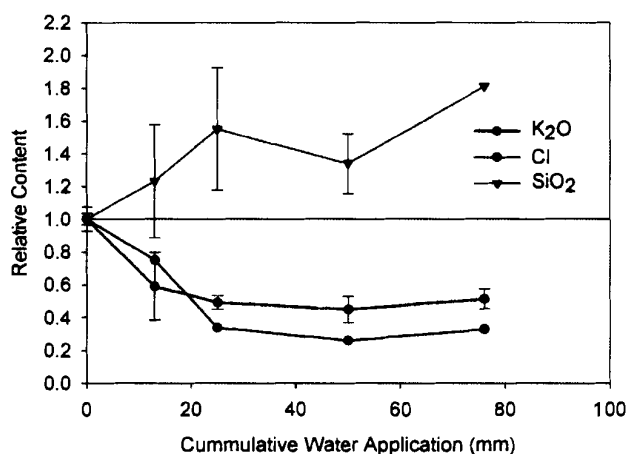


Figure 4. Response of major ash minerals to water washing.

Table 4. Chemical properties of treated straw.

	UU	WU	US	WS
	g•kg⁻¹ (dry wt. basis)			
CHO	887.91	905.99	903.34	921.12
Sulfur	1.99	1.78	2.00	1.36
Nitrogen	6.97	7.89	5.77	5.77
Ash	103.13	84.34	88.89	71.74
SiO ₂	44.36	43.79	26.30	35.16
K ₂ O	21.81	9.64	18.66	13.75
Chlorine	8.86	2.15	12.42	3.99

While straw washing easily reduced the more mobile water-soluble minerals, silica and other less mobile minerals behave much differently. In young plants, most of the minerals that comprise the straw ash are in the leaves, but after flowering, potassium, chlorine, and other mobile nutrients redistribute to stems to support grain fill (Salisbury and Ross 1992, Hocking 1994). Jacobs (1999) observed the effect of this mineral redistribution, and reported that straw stem potassium levels actually exceed levels found in other straw fractions. Thus, the separation process had little or no effect on reduction of potassium or chlorine, but the process reduced SiO₂ by 40%. This reduction can be primarily attributed to removing the sheath from the straw stem (Table 5).



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Table 5. Anatomical properties of treated straw.

	Chaff	Sheath	Stem	Node
		% Composition		
UU	3.6a [†]	11.0a	70.6a	14.3a
WU	5.9a	8.4a	80.7b	5.0b
US	3.2a	4.2b	83.7b	8.9c
WS	4.1a	5.2b	82.5b	8.2c

[†]Means within a column followed by the same letter are not significantly different (SNK method; $P < 0.05$)

Other than the chaff fraction, which included pieces/fines from all plant parts, the sheath contains the greatest weight percent of SiO_2 (Hess et al. 2003). Silica is most abundant in plant surface structures, such as the sheath, and in plant epidermal cell walls. Silica also accumulates intracellularly in specialized epidermal cells called silica cells (Salisbury and Ross 1992, Lanning and Eleuterius 1989). Unlike potassium and chlorine, once the silica is deposited in a tissue, it is not mobilized or recycled (Gali and Smith 1992). The straw sheath, which is primarily made up of once physiologically active epidermal and mesophyll cellular layers, is richer in silica than the lignified structural/vascular tissues of plant stems (Gali and Smith 1992). Thus, SiO_2 levels are primarily affected by physically separating plant parts, and the differential level in the respective plant parts is fixed (i.e., no physiological process will redistribute silica in the plant). The exception to this statement would be treatments that degrade or remove the cellular epidermal layer of plant structures, which could remove silica without removing the total plant structure (Thompson et al. 2003).

Other less mobile minerals are calcium dioxide (CaO) and iron oxide (Fe_2O_3), which are prominent in straw ash, and are known low-temperature eutectic compounds (Salisbury and Ross 1992, Jenkins et al. 1996). Like SiO_2 , the separation process rather than the washing process affected these minerals. Separation reduced CaO 30% and Fe_2O_3 33%. Reductions in sodium oxide (Na_2O), another low-temperature eutectic compound, were greatest with the separation process (63%), but washing reduced it by 45%.

While both the washing and separation pretreatments reduced overall straw ash content, the ash constituents that each process affected were different. The ultimate result was that the ratios of mineral elements could be altered based on the treatments applied (Fig. 5). These mineral ratios are, in fact, more critical to downstream combustion and staged combustion bioenergy conversion systems than the absolute levels of mineral constituents (see feedstock testing section, combustion/gasification).

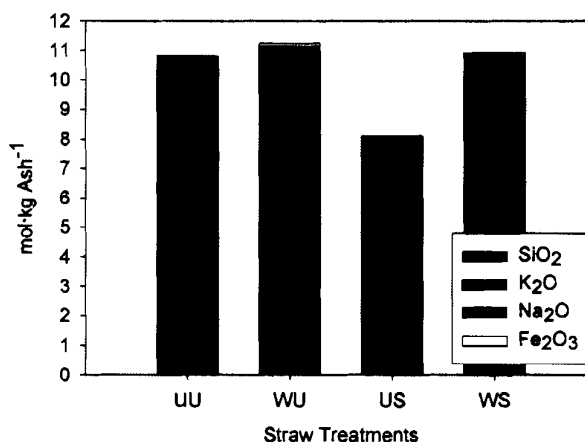


Figure 5. Ash molar concentration of alkali eutectics.

The SiO_2 - K_2O molar ratio of the UU straw was 3.19. Since the washing treatment (WU) had little to no effect on the absolute SiO_2 levels, the reduction of K_2O and other water-soluble ash minerals caused the SiO_2 wt% of ash to increase from 47.6% to 57.7%, while the K_2O wt% of ash dropped from 23.4% to 12.7%. This caused the SiO_2 - K_2O molar ratio to



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increase from 3.19 to 7.12. Separation only (US) had the opposite effect, reducing the $\text{SiO}_2\text{-K}_2\text{O}$ molar ratio to 2.21, since this treatment removed SiO_2 and not the K_2O . While performing both treatments (WS) produce the lowest absolute straw ash level (Table 4), the reduction in SiO_2 caused by the separation process offset the K_2O reduction of the washing process. This resulted in a straw ash $\text{SiO}_2\text{-K}_2\text{O}$ molar ratio of 4.01, which was only slightly improved compared to the ratio for untreated straw (UU).

The separation process reduced Fe_2O_3 by 43%, which was nearly the same level of reduction as the SiO_2 . Therefore, the $\text{SiO}_2\text{-Fe}_2\text{O}_3$ ratio was nearly unchanged as a result of the separation treatment ($\text{SiO}_2\text{-Fe}_2\text{O}_3$ ratio of 222 for UU and 230 for US). Conversely, the washing treatment actually increased Fe_2O_3 levels, which combined with other mineral losses, decreased the $\text{SiO}_2\text{-Fe}_2\text{O}_3$ ratio by 30% to 40% for WS and WU respectively. The $\text{SiO}_2\text{-Na}_2\text{O}_3$ ratio increased from 21 to 47 and 40 for the washed (WU) and separated (US), respectively. The combined treatment (WS) ratio was no better than the independent treatments ($\text{SiO}_2\text{-Na}_2\text{O}_3$ ratio of 47).

The washing process alone altered the fractional composition of the straw by reducing the node fraction and increasing the stem fraction (Table 5). Confirming that washed straw had higher stem content, we also observed that washed straw yielded 23% more stem fraction material than unwashed straw when passed through the separation process. However, while the washing treatment removed non-stem material, it didn't significantly affect sheath removal. Thus, the straw anatomical fraction composition change caused by washing did not appreciably affect mineral ratios. This supports earlier findings that node and stem fractions are relatively minor contributors to silica and total straw ash, and the sheath is a major contributor (Hess et al. 2003).

Though not quantified in this study, we observed that the washing and separation treatments altered the physical behavior of the straw in the pellet mills. Unwashed and separated (US) straw could not be pelletized as it would glass up and plug the pellet dies. However, washed and separated (WS) straw passed through the pellet machines without incident. Regardless of straw treatment, the straw could be ground into a flowable and relatively high-density form by passing it through a hammer mill with a 6.35 mm screen (Fig. 6).

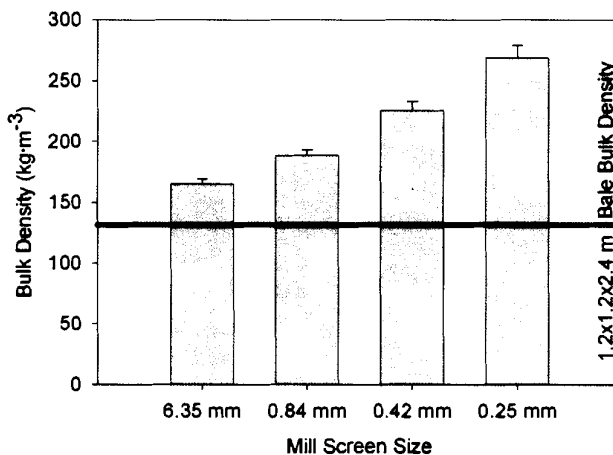


Figure 6. Bulk density of ground wheat straw.

Straw that had been hammer milled through a 6.35 mm screen worked well in pneumatic conveyance and gravity bin feed systems. This ground straw had a significant amount of fines, and generate a substantial amount of dust when handled. (To prevent fire and explosions when handling ground straw, safety equipment should be considered (Ravenet 1994).) Grinding straw to smaller particle sizes produced a more uniform product and increased the straw bulk density (Fig. 6). However, straw ground through a 6.35 mm screen is flowable and exceeds the bulk density of conventional large bales. Therefore, the benefit of these smaller particle sizes may not justify the grinding costs.



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Integration of Selective Biomass Harvest/Pretreatment into Farming Operations

Washing and separation pretreatments were effective in removing substantial amounts of alkali metals, chlorine, sulfur, calcium oxide and silica, which improved the combustion process. More importantly, the type or combination of pretreatment applied can selectively and predictably alter biomass constituents to benefit an intended bioenergy conversion end use. From a practical perspective, field washing or selective harvest of straw fractions can be done with existing farm machinery and could fit current farm enterprise practices (Fig. 7). Selective harvest machinery that can collect the straw chaff fraction is also available (REDEKOP 2004, McLeod Harvest 2004).



Figure 7. Combine and baler configured for harvesting select components of the straw residue biomass.

Task 3a – Feedstock Testing, Gasification and Combustion of Separated Straw Stems

Investigators: Judi Steciak,¹ Doug Krapas,² Richard Hess³

Performing Institutions: ¹University of Idaho, ²Energy Products of Idaho, Inc., ³Idaho National Engineering and Environmental Laboratory

The combustion and staged combustion (gasification in-bed/staged combustion above-bed) and emissions characteristics of processed wheat straws were evaluated in an atmospheric bubbling fluidized bed system located at Energy Products of Idaho's (EPI's) pilot test facility in Coeur d'Alene, Idaho. The wheat straws analyzed during the studies were arranged and processed by the University of Idaho and INEEL.

Two methods were used to treat straw to remove silicon (Si), potassium (K) and chlorine (Cl), as described in detail above (Task 1a). The first method used a pivot irrigation system to apply water to the windrowed straw left after grain harvest (washed straw). This method was effective in K and Cl removal. The second method involved mechanical separation and collection of the stems, leaving nodes, sheaths, and leaves behind in the field (separated straw). The latter method was effective in reducing the Si content of the straw. However, migration of K into stems during ripening resulted in relatively higher K:Si ratios remaining in the stems as indicated by ash oxide analysis.

Four different processed wheat straw materials were evaluated during the studies: Baseline or Unwashed and Unseparated (UU), Unwashed and Separated (US), Washed and Unseparated (WU), and Washed and Separated (WS). The various straw materials were treated in an attempt to alter the inorganic ash chemistry and affect the low-temperature eutectics that are associated with untreated wheat straw during combustion.



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A multi-day fluidized bed pilot-scale combustion and staged combustion study was conducted with wheat straw processed and furnished by the University of Idaho and INEEL (see Appendix B for facility details and photos of the gasification/combustion tests). Two separate combustion studies were conducted on each of the above material groups: standard combustion and staged combustion with gasification in the fluidized bed. Therefore, a total of eight different studies were conducted. Items of interest studied during these tests include optimization of the combustion and staged combustion processes at low operating temperatures, slagging and fouling tendencies, emissions of criteria pollutants, and carbon carryover. Raw data gathered from these tests were used to model the combustion, staged combustion, and emission parameters. The primary protocol for this project was to study the effects of various straw pretreatments on low-temperature eutectics associated with this material during fluidized bed combustion.

Fuel Test Material – Preprocessed Wheat Straw

EPI received from the University of Idaho, via INEEL and SEBS Feed and Supply, 26 supersacks of processed wheat straw. The reported net weight of the processed wheat straw was 15,360 pounds; it was supplied in the quantities noted below.

Baseline, Ground Unwashed and Unseparated (UU-GR) - 9 Supersacks

Pelletized Washed and Separated (WS-PL) - 2 Supersacks

Ground Unwashed and Separated (US-GR) - 4 Supersacks

Ground Washed and Unseparated (WU-GR) - 11 Supersacks

Fuel Inventory: 26 total Supersacks

Two separation processes were used in an attempt to alter the chemical properties of the inorganic ash fraction of the wheat straw to affect primarily the low-temperature eutectic alkali compounds: potassium and sodium. One of the processes consisted of simply washing the straw after harvesting. The washed straw was left in the field to air dry prior to shipment for further processing. The other process consisted of mechanically removing or separating the majority of the nodes contained within the straw (see detailed descriptions above, Task 1b).

The UU, US, and WU samples were all processed as a shredded, 1/2 in. minus sized material. The ground fuel was very fine in nature and quite dusty. The WS was provided in a pelletized form, 1/4 in. in diameter and approximately 1 in. minus in length. The pellets were very hard and dense and were difficult to break apart by hand. Both ground and pelletized materials were very homogeneous and dry.

Several representative samples of the wheat straw were submitted by the University of Idaho to Hazen Laboratories (Golden, Colorado) for the following analyses: Ultimate/Proximate, Elemental, Ash Fusion Temperature, and Ash Viscosity. The results of these analyses and are listed in Tables 6 and 7. The significant properties are discussed below.

The high heat energy value (HHV) reported for the processed wheat straws ranged from 7,384 to 7,891 Btu/lb (dry basis), with the baseline UU being the lowest (Table 6). The majority of this energy value is in the form of volatiles averaging approximately 75.13% (dry basis), with a lesser contribution from fixed carbon averaging approximately 16.17%. Moisture content analyses were conducted throughout the pilot studies, and ranged from 5.7 to 7.8%.

The elemental analysis identified the ash from the wheat straws to be primarily composed of silica (SiO₂), potassium oxide (K₂O), calcium dioxide (CaO), and in some cases chlorine (Cl) (Table 7). Potassium oxide, sodium oxide (Na₂O) and iron oxide (Fe₂O₃) are widely known as



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Table 6. Preprocess wheat straw fuel analysis.

Fuel Analysis				
Proximate (Wt %) As Received				
	UU	WS	WU	US
Moisture	9.63	4.66	10.01	9.89
Ash	9.32	6.84	7.59	8.01
Volatile	66.82	72.28	68.34	67.44
Fixed C	14.23	16.22	14.06	14.66
Total	100	100	100	100
Sulfur	0.18	0.13	0.16	0.18
Higher Heating Value (kJ/g)	15.52	17.70	15.56	15.90
Ultimate (Wt %) As Received				
	UU	WS	WU	US
Moisture	9.63	4.66	10.01	9.89
Carbon	40.59	44.73	41.57	41.11
Hydrogen	4.16	5.21	4.17	4.55
Nitrogen	0.63	0.55	0.71	0.52
Sulfur	0.18	0.13	0.16	0.18
Ash	9.32	6.84	7.59	8.01
Oxygen	35.49	37.88	35.79	35.74
Total	100	100	100	100
Chlorine	0.88	0.50	0.31	1.07

Table 7. Preprocessed wheat straw ash analysis.

Ash Analysis				
Elemental Analysis (Wt %)				
	UU	WS	WU	US
SiO ₂	47.60	51.40	57.70	32.84
Al ₂ O ₃	2.00	3.78	3.57	0.30
TiO ₂	0.09	0.08	0.13	0.04
Fe ₂ O ₃	0.57	0.88	1.16	0.38
CaO	5.76	6.08	6.63	4.05
MgO	2.19	2.47	2.76	1.84
Na ₂ O	2.29	1.12	1.27	0.85
K ₂ O	23.40	20.10	12.70	23.30
P ₂ O ₅	3.02	3.66	4.85	2.50
SO ₃	3.42	2.49	2.26	3.63
Cl	9.51	5.84	2.83	15.50
CO ₂	0.13	0.48	0.32	0.68
Total	99.98	98.38	96.18	85.91
Fusion Temperature (°C)				
	UU	WS	WU	US
Initial	1071	1172	1038	976
Softening	1118	1220	1129	1030
Hemispherical	1148	1307	1269	1140
Fluid	1246	1342	1424	1184



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low-temperature eutectic compounds, notorious for lowering ash-softening temperatures and causing fouling and slagging in combustion processes. The washing process alone appears to be the most effective treatment in reducing the alkali content of the wheat straw ash. A potassium (K_2O) content reduction of nearly 50% was realized with the washing process, with reductions from the baseline UU of 23.40% (dry weight basis) to the WU of 12.70% (dry weight basis). Similarly, a reduction of sodium (Na_2O) of nearly 50% was also realized with the washing process from the baseline UU of 2.29% (dry weight basis) to the WU of 1.27% (dry weight basis). The separation process appears to have little to no effect in the reduction of potassium, but did have a substantial effect in the reduction of sodium (0.85% dry weight basis). Subsequently, the Washed and Separated (WS) process had a slight effect in the reduction of potassium (20.10% dry weight basis) and relatively significant effect in the reduction of sodium (1.12% dry weight basis). Iron oxide (0.38 to 1.16% by weight) and sodium oxide (0.85 to 2.29% by weight) were present in relatively small quantities. However, most important is the effect of the various processes on raising the ash fusion temperatures. In all cases, the washing and separation processes were effective in significantly increasing the Ash Fusion Temperatures in both the oxidizing and reducing atmospheres.

The chemical characteristics of the processed wheat straws of interest for formation of criteria pollutants are primarily the ash, sulfur, nitrogen and chlorine. The ash content reported in the ultimate/proximate analysis ranged from 7.17% to 10.31% (dry weight basis), resulting in modest ash collection rates from the cyclone and baghouse. Both the washing and separating processes were effective in reducing the ash from the baseline UU value. The low sulfur content of the processed straws, averaging 0.18% (dry weight basis), resulted in insignificant or non-detectable levels of sulfur dioxide emissions. The average nitrogen content of 0.66% (dry weight basis) contributed to the formation of NO_x emissions. The chlorine content of the straws, averaging 0.75%, is relatively high and will contribute to the formation of hydrochloric (HCl) acid gas emissions during the combustion process.

Gaseous KCl reacts with SO_3 and O_2 to form K_2SO_4 (potassium sulfate), releasing Cl. Because this reaction occurs in flyash deposits, there is a greater than normal concentration of Cl at metal surfaces and a less than normal concentration of oxygen. This chlorine forms compounds with the chromium in the steel alloy. The chlorine-chromium compounds are volatile at boiler space temperatures. As the vapor reenters the gas stream, oxygen combines with the chromium. Chlorine is released for further reaction. At temperatures above 520°C, corrosion is greatly enhanced. High boiler temperatures are needed to maximize power plant efficiency. A boiler operating at colder conditions to mitigate corrosion from high K and Cl levels in biofuels must operate at reduced efficiency (derated).

Four ash samples were tested by differential thermal analysis (DTA) to determine the thermal activity as the samples were heated from ambient temperature to about 1100°C. For this procedure, the selected samples and an inert reference material (Al_2O_3) were added to separate platinum cups and then positioned on a matched pair of Type-R thermocouples. A furnace tube isolated the atmosphere surrounding the sample and Al_2O_3 , which was purged continuously with nitrogen. The sample and reference materials were heated simultaneously at 20°C/min to the limit of 1100°C. The temperature difference between the sample and Al_2O_3 was recorded on a thermogram, which shows endothermic (below baseline) and exothermic (above baseline) deflections. Endothermic occurrences (heat consuming) are associated with phase changes, decomposition, and melting. Exothermal occurrences (heat releasing) are related to combustion and oxidation.

At the completion of each DTA run, the sample residue was inspected. The residue from each sample except the WUGR ash appeared to have melted. The residue from the WUGR ash was



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a very hard pellet that was approximately 1/8 the volume of the original sample. All of the samples had an initial endothermic deflection that occurred between 640 and 665°C. Second and third endotherms were also apparent for some of the samples. The actual temperatures for the thermal activity are summarized in Table 8.

Table 8. Temperatures of thermal activity.

Sample		Temperature (°C)			Melted Residue?
		Endotherm 1	Endotherm 2	Endotherm 3	
Unwashed, unseparated	UUGR ash	640	694	959	Yes
Unwashed, unseparated	UUGR-repeat	639	697	960	Yes
Unwashed, separated	USGR ash	665	724	971	Yes
Washed, unseparated	WUGR ash	649	Na	957	No
Washed, separated	WSPL ash	657	Na	950 ?	Yes

Unwashed = not exposed to pivot irrigation in the field; Washed = exposed to pivot irrigation; Unseparated = entire straw residue collected; Separated = nodes and sheaves separated; Na = not apparent; ? = possible endothermic temperature

A sample that has melted will generally exhibit an exothermic deflection as the sample temperature cools through the melting temperature zone. There were no exothermic deflections apparent for these samples during the cooling cycle. Sample melting was determined by visual inspection of the residues. Endothermic deflections that occurred below 700°C are not attributed to melting for these samples. This determination was based on visual observation of the sample as the temperature increased.

Based on the DTA analysis, only the washed and unseparated straw had unmelted residue. This is consistent with the combustion and gasification tests wherein the WU straw was the only sample that did not need addition of limestone to the fluidized bed to prevent agglomeration.

Bed Material

A CE Minerals Mulgrain 47, size 22S was selected for the fluid bed media during the tests. This bed media is a calcined mullite product with high thermal shock resistance and abrasive qualities desirable for optimum fluid bed operation. The material size, 20 x 50 mesh, is selected based on finer fuel particle size and fluidization properties at the predicted operating conditions.

Limestone

Two different limestones were evaluated during the studies to minimize slagging in the combustor, one supplied by Continental Lime Company (Cricket Mountain Site) and the other by the Chemical Lime Company (Apex, NV site). Both limestones were comprised of a minimum 95% lime (CaO) content.

Gasification and Combustion – Preprocessed Wheat Straw

Washing without separating had the greatest effect in reducing the potassium content of the inorganic ash fraction of the wheat straws, with a reduction from 23.40% (by weight) for the baseline UU to 12.70% (by weight) for the WU. The sodium content of the ash (sodium is also a well-known low-temperature eutectic) was also reduced from 2.29% for the baseline to 1.27% for the WU. The overall affect of this alkali reduction is significant - 3.59 lbs. of Alkali/MBtu for the Baseline UU to 1.52 lbs. of Alkali/MBTU for the WU. This alkali reduction resulted in an increase in the overall fusion temperature of the ash of over 200°F in an oxidizing atmosphere and over 300°F in a reducing atmosphere. The US and WS processes were less effective in reducing the alkali content of the ash.



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The UU, US, and WU materials were provided as a shredded material less than 1/2 in. in length, and the WS was provided in a 1/4 in. diameter pelletized form less than 1 in. in length. The shredded material was conveyed pneumatically directly into the fluidized bed region of the combustor, and the pelletized material was gravity fed above the bed. The decision to process the materials in this manner was due to material handling and combustion difficulties raised during a prior pilot study with 6–18 in. lengths of straw.

Ten days of testing were required to evaluate both the combustion and staged combustion processes for all four materials. The primary test protocol was to attempt to successfully burn all four samples of wheat straw in both combustion and staged combustion modes and compare the results of each. The protocol was to include optimization of combustion temperatures, limestone feed rates, overfire air and flue gas recirculation rates, emissions, and all other combustion parameters.

In standard combustion modes, fuel was fed to the system at a reduced rate corresponding with the choked bed area. Typically with this arrangement, all of the air necessary for complete combustion was provided as under-fire or fluidizing air. In staged combustion, fuel was fed at the rated capacity of the combustion system (3.0 to 4.0 MBtu/hour). The abundance of fuel in the choked area of the bed resulted in gasification in the fluidized bed zone. The balance of combustion and attemperation air was added in stages in the vapor space as overfire air (OFA) and flue gas recirculation (FGR).

Pneumatic in-bed feeding of the shredded wheat straws (UU, US and WU) provided for virtually uninterrupted and homogeneous conveyance of the fuel. Plugging of the pneumatic conveyance line occurred on several occasions, but these were primarily due to foreign materials entering the system. At the higher fuel feed rates required for staged combustion, approximately 25% of the straw was diverted to the above-bed gravity feed system to reduce the load on the in-bed rotary feeder.

The in-bed injection of the finer, high-energy, low moisture content fuel provided for excellent in-bed heat release in both combustion and staged combustion modes of operation. A minimum temperature of 1,500°F was required in the vapor space to maintain levels of carbon monoxide (CO) below 100 ppm_{dv} (ppm on a dry volume basis). Operations below this threshold temperature resulted in erratic levels of CO emissions, with spikes in excess of 2,000 ppm_{dv}.

The Washed and Unseparated (WU) wheat straw tests were conducted without the use of limestone due to the low alkali content and subsequent increased ash fusion temperatures resulting from the washing process. Very efficient combustion was observed throughout both the combustion and staged combustion tests with emissions of CO consistently below 25 ppm_{dv}. Some slag formations were observed at the end of the staged combustion studies, but only in the location of the uncontrolled temperature zone at the OFA injection levels.

The Washed and Separated (WS) wheat straw was provided in a pelletized form approximately 1/4 in. in diameter with varying lengths less than 1 in. The larger size and density of the pellets required conveyance to the combustor with the above-bed gravity-feed system. The mass and energy density of the pellets was high, resulting in significant in-bed heat release. The release of energy in-bed was so high that during combustion modes, bed temperatures exceeded 1,700°F with very low vapor temperatures. However, CO emissions were also low, indicating nearly complete combustion was taking place in-bed.

The WS pellets performed well in both combustion and staged combustion modes. However, the large particle size, high-energy value and in-bed heat release caused some significant



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problems during shutdown of the system when in staged combustion mode. When the fuel supply was shut-off at the end of the study day, as the in-bed fuel began to be consumed the air/fuel ratio began to increase. The process continued to increase along the gasification curve towards stoichiometry and high temperatures in excess of 2,000°F (maximum output range of thermocouples/transmitters) were encountered. During these episodes, the bed became glassed over (solidified), and numerous thermocouples were destroyed. This required the unit to be shutdown, cooled-off, the bed drained and cleaned of clinkers, thermocouples repaired, and recharged with new bed media.

Hindsight evaluation of the above scenario has resulted in the following operation and shutdown procedure to prevent this occurrence in full-scale system application. Sufficient fluidizing air must be provided during the normal operation of the stage combustion process to allow for the reduction of airflow as the fuel supply is shut off. As the fuel inventory is being consumed, the airflow is reduced to maintain the same air/fuel ratio. At a point where fluidizing air is at a minimum, and the bed temperatures begin to increase, the airflow should be increased significantly to "jump the curve" from gasification into combustion. Water injection above the bed would also be recommended for these transitions from staged combustion to combustion.

The reduced bed size (1 ft-4 in. x 1 ft-4 in.) required for staged combustion in the pilot-scale system resulted in a greatly reduced bed media volume, creating several operating difficulties. In order to maintain bed cleanliness, rapid change-out of the bed was necessary to minimize clinker formation and build-up. During bed change-out cycles, a significant fraction of the active bed media was removed. The re-injection of cooled bed media into the reduced active bed caused a rapid decrease in bed temperatures and subsequently erratic emissions of CO. Shortening the "open" interval time of the slide gate and increasing the bed change-out rate to balance the system minimized the effect of this phenomenon. This effect would not exist in a full-scale system design, due to the large inventory of bed media.

The lower operating temperatures, rapid bed change-out with a reduced bed volume, radiant losses to the refractory due to the short operating period, high limestone feed rates and the low density of the ground wheat straw all combined to cause erratic emissions of CO during periods of the study. When all of the above factors were optimized during each test period, emissions of CO could be maintained below 50 ppmv. In full-scale application with steady-state operation many of the above factors would not be applicable, and CO emissions less than 50 ppmv would be readily achievable.

Emissions of other criteria pollutants proved to be more favorable due to the chemical characteristics and the homogeneous nature of the fuel. The low sulfur content of the wheat straw and the calcium oxide (CaO) in the ash resulted in very low or non-detectable levels of sulfur dioxide emissions. The addition of limestone for fouling and slagging control also provided an abundance of CaO, further reducing any potential of SO_x emissions.

During steady-state operation, emissions of nitrogen oxides (NO_x) ranged from a low of 40 to a high of 200 ppmv depending upon the chosen operating parameters and the wheat straw sample. The pelletized WS wheat straw produced the lowest NO_x emissions, likely due to the significant in-bed heat release and the minimal use of overfire air (OFA) and FGR. The ground wheat straws (UU, US and WU) produced the higher levels of NO_x emissions due to the significant use of OFA and FGR to control vapor temperatures and complete combustion. The introduction of OFA and FGR into the vapor space during staged combustion resulted in higher flame temperatures, and subsequently higher levels of NO_x emissions.



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Internal inspections of the combustion system were conducted throughout the tests for fouling and slagging deposits. The studies conducted on the ground wheat straws typically revealed a minor build-up of fine fibrous straw remnants, resembling carbon black, in areas of the vapor space on ledges, and thermocouples. The fibrous build-up was ash like in appearance and did not pose any significant concern to long-term operations. Slag deposits were discovered on several occasions at the overfire air injection port locations. These deposits were created only during staged combustion in the high flame temperature zone of the OFA ports where combustion/attenuation air was injected for combustion of the volatile gases produced from the bed. Primarily this slag deposit was observed during the WU studies without the use of limestone as mentioned above. The WS pellets also contributed to "glassing" of the bed on two occasions during staged combustion as discussed above.

Throughout the studies the bed change-out system was operated at a significant cycle rate to remove any clinker formations. During the test runs with all wheat straw control groups, a significant amount of small clinker formations were removed continuously from the bed. However, these small agglomerations did not pose any threat to the operation of the system as long as they were continually removed. The use of an efficient bed change-out system will be crucial to the long-term operation of a full-scale production facility to continually remove these small formations.

This study has successfully achieved the results of the test protocol. Based on the results of this study, any of the processed wheat straw samples could successfully be burned in a fluidized bed application in either combustion or a staged combustion process. With consideration of the various design modifications and operating conditions developed from this study, EPI's fluidized bed technology could meet all design, energy, and environmental demands imposed for full-scale application.

Results – Engineered Fungal Preprocessing Wheat Straw Storage Systems for Straw-Thermoplastics Production

The principal barriers to straw utilization for straw-thermoplastic composites are resin penetration and resin consumption. Resin penetration is limited by the physical barrier presented by the cuticle and underlying epidermis on the external surface of the straw, and by the lignin-hemicellulose matrix in the internal vascular layer. Resin consumption is increased because the resin does not bind well to the cuticle, and because fines, created when the nodes and leaves are ground, have high surface areas and require much more resin. Since straw thermoplastics can contain as much as 50 – 70 wt% straw, resin binding and performance are of the utmost importance, as has been shown in wood-plastic composites (Wolcott and Englund 1999, Sanadi et al. 1997).

We chose *P. ostreatus* for our experiments because it is a member of the white-rot fungi, known for their ability to degrade lignin. The term "white-rot" originates from the visual appearance of the degraded wood which loses its brown coloration with the removal of lignin. Lignin removal or modification by white-rot fungi or their derived enzymes has previously been applied in pulp production (Messner and Srebotnik 1994, Camarero et al. 1998), environmental bioremediation (Aust 1990), bioremediation of preservative-treated waste-wood (Lamar and Dietrich 1992, Lee et al. 1992a and b), particle- and fiberboard manufacturing (Kharazipour et al. 1998, Felby et al. 2002) and as treatment for upgrading animal feeds (Zadrazil 1977, Akin et al. 1993, Hatakka et al. 1989).



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Treatment of wheat straw with *P. ostreatus* is expected to cause a limited degradation of the waxy cuticle, lignin and hemicelluloses in the straw without much cellulose removal (Valmaseda et al. 1991, Moyson and Verachtert 1991, Gamble et al. 1996). Degradation of these selected components of wheat straw is expected to (1) improve adhesion between the straw and plastic, and thereby the mechanical properties of the composite material; and (2) possibly reduce the amount of polyethylene required in the production of high-density polyethylene-based composite materials, and hence, the cost of the composite product.

One principle governing limitation of the selective removal of lignocellulosic material is the potential loss of thermostability of the degraded product. The primary criterion used in the selection of a thermoplastic for production of a wood- or straw-plastic composite is that the melting or softening temperature of the thermoplastic is less than the thermal degradation temperature of the wood- or straw-filler (ca. 210°C for wood and undegraded wheat straw) (Wolcott and Englund 1999). This thermal criterion restricts the polymer class of potential thermoplastics to polyolephins (Wolcott and Englund 1999). Treatment of wheat straw with a white-rot fungus which selectively degrades lignin, the thermally most stable component in lignocellulosic materials (Fengel and Wegener 1989), may reduce the thermal stability of the degraded product below 210°C, thus precluding the use of polyethylene, and polyolephins in general, in the production of a thermoplastic composite.

An additional objective of our research, not part of the original project proposal, was to investigate the effect of sterilization of wheat straw prior to inoculation of the substrate with *P. ostreatus*. Yadav (1988) demonstrated that processing of straw under farm (unsterile) conditions resulted in growth of contaminating fungi, low specific growth rate of the inoculated fungus and poor substrate utilization. In addition, it has been shown that sterilization of wood chips prior to application of fungal inoculum in biopulping is necessary to achieve consistent results when using fungi for delignification (Scott and Akhtar 2001).

Task 2a – Fungal Preprocessing of Straw Residue, Laboratory Optimization of Selective Straw Degradation

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Acknowledgements: The authors thank Dr. Stephen Aust and Paul Swaner at Utah State University for maintaining and supplying the fungal cultures for inoculum production; and Robert S. Cherry, Karen Miller, Peter G. Shaw, Neal Yancey, and Jeff Parry at the Idaho National Engineering and Environmental Laboratory.

This task focused on determining whether *P. ostreatus* could be competitive with indigenous organisms in a distributed system treating unsterilized wheat straw stems. In addition, another goal of the task was to determine the expected variations in straw composition with significant variations in initial moisture content and fungal inoculum size.

The physical barriers to resin penetration are the same physical barriers that limit access of cellulase enzymes to the cellulose fibers when producing glucose from straw cellulose for fermentation to ethanol or for production of fuels and chemicals. Much of the research on removal of the lignin-hemicellulose barrier to date has been on conversion of the cellulose to glucose, since glucose can easily be fermented to ethanol using common yeasts (Marsden and



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Gray 1986). Dilute acid hydrolysis of the cellulose to glucose lowers fermentable carbohydrate yields due to thermal decomposition of xylose to furfural and glucose to hydroxymethylfurfural (Converse et al. 1989). Thus, much work has been done on the use of cellulase enzymes, since they are specific for cellulose, form only glucose, and the hydrolysis is performed at mild temperatures. However, cellulases are relatively large enzymes, and cannot fit through most of the spaces in the intact vascular layer of straw (Cowling and Kirk 1976, Thompson et al. 1992). Thus, physical, chemical, and thermal pretreatments are employed to increase the penetration of cellulases into this lignocellulose matrix (Marsden and Gray 1986, Cowling and Kirk 1976, Fan et al. 1982). Many pretreatments have been developed, including acids (Knappert et al. 1980, Goldstein et al. 1983), alkalis (Playne 1984, Weimer et al. 1986), organosolvents (Avgerinos and Wang 1983), steam explosion (Playne 1984, Taylor 1981), physical treatments, and others. Although effective, the pretreatments are costly, negatively affecting the economics of utilization. Pretreatments with white-rot fungi, which have been shown to completely degrade lignocellulose, increase glucose yields without significant capital or energy intensive steps (Hatakka 1983). The principal drawbacks to centralized white-rot fungal pretreatments are that the process footprints are large and that treatment times are often eight weeks or more, much too long for use at large industrial facilities (Hatakka 1983).

Since lignin and hemicellulose limit resin penetration just as they limit cellulase penetration into the matrix, these pretreatments would be expected to improve resin penetration as well. Indeed, steam explosion has been investigated to remove lignin and hemicellulose to allow better resin penetration and adhesion (Avella et al. 1998). While significant improvement in interfacial adhesion was seen in steam exploded broom fibers, the extensive physical damage to the fibers imparted by the steam explosion process eliminated any mechanical property improvements. Of course, steam explosion pretreatment, whether for better resin penetration or for better penetration of cellulases, requires significant capital equipment and energy input. Thus, an inexpensive, low capital, low energy-input treatment, such as a fungal pretreatment, that removes the cuticle, lignin, and hemicellulose would be ideal. Physical removal of straw components that form fines (Hess et al. 2003) would also help to reduce resin consumption. Combining physical removal of fines (Hess et al. 2003) and fungal treatment into a distributed process that could be done onsite at a small scale would minimize the land area required and minimize capital and energy inputs.

White-rot fungi remove lignin using extracellular peroxidase enzymes, attacking the lignocellulose matrix by growing into the cell walls, where they secrete extracellular cellulases, hemicellulases, and peroxidases (Boominathan and Reddy 1992). Ligninolytic enzymes are produced in secondary metabolism under conditions of carbon or nitrogen deprivation (Boominathan and Reddy 1992). The degraded lignin is not used as a growth substrate, but is removed to open up the matrix to cellulases and hemicellulases so that over time near-complete degradation is possible (Blanchette et al. 1989). While cellulose and hemicellulose are the principal growth substrates for white-rot fungi (Boominathan and Reddy 1992), some white-rot fungi, including several *Pleurotus* species, attack straw lignin and hemicellulose without much cellulose removal (Valmaseda et al. 1990, Moyson and Verachtert 1991). There is also evidence of degradation of the cuticle during degradation by white-rot fungi (Gamble et al. 1996). Once through the cuticle, the fungi possess the necessary cellulases and hemicellulases to degrade the epidermal layer, allowing access to the vascular layer from the outer surface of the residue. Thus, in a single degradation step, white-rot fungi could potentially upgrade the straw to a more desirable feedstock for straw-thermoplastic composites. That is, a selectively upgraded straw product should have a higher cellulose content, and partial degradation of the vascular hemicellulose and lignin, the cuticle layer, and the epidermis should allow better penetration by resins used in thermoplastic extrusion processes.



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Wheat Straw & Fungal Sources & Materials

Wheat straw (Westbred 936), a hard red spring variety, was obtained from Grant 4-D Farms (Rupert, ID). All straw utilized in this study was produced during the year 2000 cropping season. Twenty large bales of Westbred 936 (1.2 m × 2.4 m bales (4 ft × 8 ft)) were produced and stored in a stack at the side of the field at Grant 4-D Farms. Only the protected center bales from the interior of the stack were used for the studies. This was done to minimize the effects of exogenous nitrogen sources (i.e. bales touching soil) and water (i.e. bales on the outer periphery of the stack), which were important variables because we intended to limit nitrogen and vary water in the fungal degradation tests. To better handle the straw for the laboratory studies, the large bales were rebaled as needed into smaller 0.61 m × 1.2 m (2 ft × 4 ft) bales containing about 22.7 kg (50 lb) each, and placed in covered storage. To remove the plant components that are the sources of high surface area fines (leaves, sheaths, nodes, and fines), the straw was rethreshed before use as described by Hess et al. (2003). Only the separated straw stems were used in the laboratory studies.

Wheat straw ash content was determined as follows. At least 1 g of dry stems, ground to 60 mesh, was ashed in a muffle furnace at 650°C for 18-24 h. Ash content was calculated by weight difference. Carbohydrate and lignin compositions of untreated and treated straw samples were determined by quantitative saccharification using the method of Saeman et al. (1945). Two aliquots of each sample were analyzed by quantitative saccharification for each of the three replicate columns at each condition, for a total of 12 independent measurements of each composition. Carbohydrate analyses were done by high performance liquid chromatography (HPLC) using a BioRad HPX-87P carbohydrate column as previously described (Thompson et al. 1992). The acid-insoluble fraction from the quantitative saccharification was ashed at 650°C, and Klason lignin with extractives was calculated by weight difference. The amounts of glucan and xylan degraded per 100 g of initial weight were calculated by mass balance assuming the sum of lignin, ash, and extractives remained constant. The percent conversions of glucan and xylan (ΔG and ΔX , respectively) were then calculated by dividing by the initial basis weight of each and multiplying by 100.

Pleurotus ostreatus NRRL 2366 was chosen for use in the fungal degradation tests based on its ability to selectively degrade the noncellulose components of wheat straw (Hadar et al. 1993, Lindfelser et al. 1979). It was obtained from the Northern Regional Research Laboratory (NRRL) (Peoria, IL). Stock cultures were maintained at Utah State University on agar slants at room temperature containing 20 g/L of YM agar (Difco, Detroit, MI) and the following trace minerals—0.02 g/L of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.004 g/L of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.002 g/L of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.002 g/L of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, and 0.001 g/L of $(\text{NH}_4)_2\text{MoO}_4 \cdot 4\text{H}_2\text{O}$. Stock cultures were subcultured every 2 weeks. Stock mycelial inocula were produced at Utah State University as follows. Fungal mycelia were transferred from the maintenance slants to 100 mL of 20 g/L YM broth (Difco) using a sterile loop and grown in agitated culture for 2 to 3 d at room temperature and 180 rpm. This culture was transferred to a sterile Fernbach flask containing 1 L of 20 g/L YM broth with trace minerals as described above, and agitated for 4 d at room temperature and 180 rpm. The fungal pellets were harvested by light centrifugation (380 ×g) in sterile centrifuge bottles, transferred to sterile bottles with sufficient spent medium to submerge the pellets, shipped under refrigeration to the INEEL, and stored at 4°C until use (typically 2 to 3 weeks or less).

Nitrogen Consumption Experiments

Since it would be uneconomical to sterilize the straw for use in distributed systems, unsterilized straw is preferred and thus the inoculated *P. ostreatus* must be able to out-compete or overtake



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the indigenous microbial population. White rot fungi are late colonizers in nature and dominate under conditions of nitrogen limitation (Kirk and Farrell 1987). Thus, minimizing the amount of nitrogen carried over to the straw in the inoculum could allow the inoculated fungi to more quickly overtake the indigenous microbes, minimizing or eliminating the need for sterilization. The nitrogen consumption tests were designed to estimate the initial nitrogen level required to produce a suitable amount of biomass for inoculating the straw while minimizing the amount of nitrogen remaining in the culture medium.

In these experiments, approximately 500 mL of wet fungal pellets of *P. ostreatus* were transferred to a sterile blender, and 500 mL of medium were added. Media tested included YM broth with trace minerals (as above), and a nitrogen-limited medium containing 20 g/L glucose, 1.0 – 3.5 g/L yeast extract, and trace minerals (as above). The mixture was blended for 2 min, producing a slurry of finely chopped mycelial fragments. The optical density (OD) at 550 nm was determined for dilutions of this slurry using a standard UV/Vis spectrophotometer. The undiluted slurry was then inoculated to 1.0 OD into fresh nitrogen-limited medium in sterile shake flasks and incubated for 14 days at 30°C, 135 rpm. Replicate flasks were sacrificed periodically, and the fungal pellets were washed with distilled water and separated from the liquid by centrifugation for 10 min at 26,892 x g. Fungal biomass was measured gravimetrically after drying for 48 hours at 105°C. Total Kjeldahl Nitrogen (TKN) was measured as previously described (American Public Health Association 1989).

Preparation of Fungal Inoculum for Straw Upgrading Test Columns

Unsterile straw stems were used in all experiments because sterilization of large quantities of straw for construction of large windrows would be uneconomical and impractical. For this strategy to be effective, it was necessary that the inoculated fungus be able to compete with the indigenous organisms in the straw. Since white-rot fungi dominate in nature under conditions of nitrogen deprivation (Kirk and Farrell 1987), experiments were performed to determine inoculum production conditions necessary to limit nitrogen addition during inoculation of the straw with the fungus. The most suitable conditions for minimization of nitrogen remaining in the culture and production of biomass in the inoculum, as determined over the course of the tests, were used. C/N ratios in the N-limited media tested ranged from about 29 to 89, and were adjusted by adding yeast extract to 20 g/L glucose solutions. The nitrogen-limited medium finally utilized for straw stem inoculum production contained 3.0 g/L yeast extract, for a C/N of 32.6. Mycelial inocula for inoculation of straw stems were produced in this nitrogen-limited medium, using as inoculum the fungal pellets produced at Utah State University in YM broth.

The column inoculum cultures were cultured and harvested in a manner similar to that used for the nitrogen consumption experiments, except that the cultures were harvested between days 5 and 7, and the mycelial pellets were homogenized in the spent medium, without centrifugation. Approximately 500 mL of wet fungal pellets of *P. ostreatus* grown at Utah State University in YM broth were transferred to a sterile blender and blended for 2 min, producing a slurry of finely chopped mycelial fragments. The optical density (OD) at 550 nm was determined for dilutions of this slurry using a standard UV/VIS spectrophotometer. The undiluted slurry was then inoculated to 1.0 OD into the fresh nitrogen-limited medium in sterile shake flasks and incubated for 5 to 7 d at 30°C, 135 rpm. The fungal pellets in the inoculum cultures were then transferred with the spent medium to a sterile blender and blended for 2 min. The OD at 550 nm was determined for dilutions of this slurry and the concentration of biomass was estimated from a previously measured calibration. The undiluted slurry was transferred to a sterile hand-pump garden sprayer for addition to the straw stems. No extraordinary measures were taken beyond this point to maintain sterility, except for using initially sterile equipment.



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Initial Small-Column Wheat Straw Upgrading Experiments – Determination of Minimum Competitive *P. Ostreatus* Inoculum Size

Because we intended to standardize the inoculum, it was desirable to know the exact amount of fungal inoculum added to the straw. In addition, it was desired to minimize heterogeneity in the treated straw by initially evenly distributing the fungus over the entire surface of the straw. To accomplish this, *P. ostreatus* was added to the straw by spraying a liquid suspension of homogenized mycelia onto the straw with mixing. Assuming the availability of a viable stabilized homogenized mycelial inoculum, this would also be a more cost effective method for inoculating straw piles in the field than using a tumbler to mix solid inoculum into untreated straw. However, because spraying is not a commonly used inoculation method, there was no obvious initial range of fungal inoculum to test. Since 10 wt% of solid inoculum is often used in soil bioremediation to inoculate with white rot fungi using the solid inoculation method (Dr. S.D. Aust, Personal Communication, Utah State University), we arbitrarily assumed that the solid inoculum contained 1% of its weight as dry fungal biomass and chose the range 0 – 1 wt% dry fungal biomass for the initial column experiments.

The fungal pellets in the inoculum cultures, produced immediately before inoculation of the straw columns, were transferred with the spent medium to a sterile blender and blended for 2 min. The OD at 550 nm was determined for dilutions of this slurry and the concentration of biomass estimated from a previously-measured calibration. The undiluted slurry was transferred to a sterile standard hand-pump garden sprayer for addition to the straw stems. No extraordinary measures were taken beyond this point to maintain sterility, except for using initially sterile equipment.

Air-dried straw stems (150 g dry weight) were spread onto a clean, dry tray in a thin layer, and the inoculum was sprayed onto the stems, with frequent mixing of both the inoculum and the stems. Enough inoculum was added to reach the desired initial level of fungal inoculum in the stems. Periodically during inoculum addition, a fan was used to blow air across the tray of inoculated straw to evaporate excess water, with frequent mixing of the straw. After the desired amount of inoculum was added, additional sterile distilled water was sprayed onto the straw as needed to reach the desired initial moisture content for the particular experiment. A separate sample of the well-mixed inoculum slurry was then added to a tared bottle and dried at 105°C to determine the actual biomass concentration of the slurry. The initial moisture range to be tested was chosen based on empirical evidence from compost biofiltration and soil bioremediation using white rot fungi. First, a gravimetric moisture range of up to 70% (41% on a wet basis) has worked very well in compost biofiltration experiments degrading volatile organic compounds (VOCs) at the INEEL (Cherry, and Thompson 1997, Kastner et al. 1999). In addition, it has been shown that white rot fungi seem to grow and compete best with indigenous microbes in soil at moisture levels at or below 0.5 g H₂O/g (Stahl and Aust 1998). Thus, since it was desired to have the inoculated fungus successfully compete with the indigenous microbes, the initial gravimetric moisture range was chosen to bracket these values, at 0.40 – 0.70 g H₂O/g dry stems (40 – 70% on a dry basis).

The inoculated straw was added to clean, initially-sterile glass columns fabricated from glass process pipe. The airtight columns were comprised of a 12 in. section of 3 in. i.d. Pyrex process pipe with 2 in. i.d. reducers at each end (ACE Glass, Inc., Vineland, NJ), and Teflon® end-caps. The columns were prepared in triplicate with approximately 50 g dry weight of inoculated stems in each column. The loaded columns were supplied with humidified oil-free instrument air at 15.5 psig and a flow rate sufficient to turn over the air in the system once per day. Approximately 2.5 g dry weight of straw was sampled from the top and bottom of each column initially and



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approximately every 3 – 4 weeks thereafter. The samples were dried at 105°C overnight and ground to 60 mesh in a Wiley mill for compositional analysis.

Determination of Dominance of Inoculated Fungus

Pleurotus species, including *P. ostreatus*, are somewhat selective for hemicellulose and lignin rather than cellulose (Saeman et al. 1945, Hadar et al. 1993, Martinez et al. 1994). Because a mixed culture of indigenous microbes can degrade the most easily accessible fractions of both cellulose and hemicellulose, the indigenous microbes would not be expected to show significant selectivity for specific polysaccharides. Thus, the mass balances of cellulose and hemicellulose could potentially be used to determine whether the inoculated *P. ostreatus* was dominant, by comparing the relative amounts of degradation of cellulose and hemicellulose. Thus, a column that showed similar degradation of both cellulose and hemicellulose (cellulose/hemicellulose ratio similar to that for undegraded straw) was said to be dominated by indigenous microbes. Conversely, a column that showed significantly greater hemicellulose degradation than cellulose degradation (significantly increased ratio of cellulose/hemicellulose) was said to be dominated by the inoculated *P. ostreatus*. The performance measures chosen for the inoculum competition column tests were the relative changes in cellulose and hemicellulose after degradation (expressed as the cellulose/hemicellulose ratio), as well as overall hemicellulose removal. Thus, cellulose, hemicellulose, lignin/extractives, and ash were measured, and the mass balances from day 0 to the sample date were compared. It is true that lignin removal could be used separately or together with cellulose/hemicellulose ratio as an indication of *P. ostreatus* activity. However, routine measurement of extractives would be necessary to discern changes in lignin and extractives. Since the cellulose/hemicellulose ratio was believed to be a good indicator of the relative activity of the inoculated white rot fungus versus the indigenous microbes, it was decided not to measure the extractives for treated straw samples until the final conditions were chosen. Thus, lignin is expressed as "lignin with extractives" in this report.

Subsequent Small-Column Wheat Straw Upgrading Experiments – Determination of Variations in Final Straw Compositions with Moisture Content and Inoculum Size

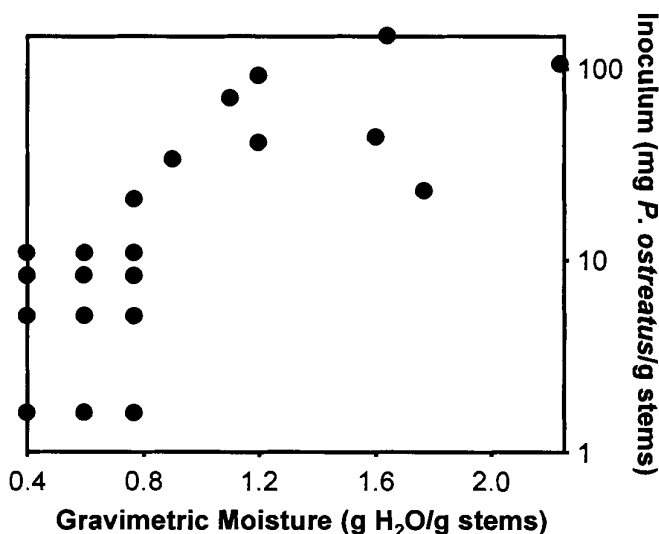


Figure 8. Combinations of inoculum amount and moisture content tested.

Combinations of inoculum amounts and moisture contents tested in this exploratory study are shown in Figure 8; controls lacking inoculum were also conducted during the initial testing phase at 0.4 – 0.77 g H₂O/g stems and are not shown in Figure 8. The initial upgrading tests performed in the small test columns are represented by the 12 points in the lower left-hand corner of Figure 8. When the initial tests indicated that higher inoculum was needed for better selectivity and that higher moisture was needed for faster degradation, parameter testing moved to the combinations plotted in the upper middle and right-hand corner of Figure 8.



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Air-dried straw stems (150 g dry wt at ca. 9 – 13 wt% moisture) were weighed onto a clean, dry tared tray and spread in a thin (5 cm) layer. The homogenized mycelial inoculum slurry was then sprayed onto the stems, with frequent mixing of both the inoculum and the stems. Sufficient inoculum was added to reach the desired initial level of fungal inoculum in the stems. Periodically during addition of inoculum, a fan was used to blow nonsterile air across the tray of inoculated straw to evaporate excess water, with frequent mixing of the straw. After the desired amount of inoculum was added, additional sterile distilled water was sprayed onto the straw as needed to reach the desired initial moisture content for the particular experiment. A separate sample of the well-mixed inoculum slurry was then added to a tared bottle and dried to constant weight at 105°C to determine the actual biomass concentration of the slurry. In addition, several small samples of the inoculated stems were transferred to tared bottles and dried to constant weight at 105°C to determine the actual moisture content of the inoculated stems.

The inoculated straw was added to clean, initially sterile columns fabricated from glass process pipe as described above. The columns were prepared in triplicate with approximately 50 g dry weight of inoculated stems in each column. The loaded columns were supplied with humidified oil-free instrument air at 193 kPa (28 psia) and a flow rate sufficient to turn over the air in the system once per day (about 10 mL/min). Approximately 2.5 g (dry wt) of straw was sampled from the top and bottom of each column initially and approximately every 3 to 4 weeks thereafter for 12 weeks. The samples were combined, dried to constant weight overnight at 105°C and ground to 60 mesh in a Wiley mill for compositional analyses.

Larger-Scale Laboratory Treatment in Drums

Straw stems were first treated for 6 weeks in small columns with *P. ostreatus* at 40 mg *P. ostreatus* /g stems and 1.6 g H₂O/g dry stems, as described above. These stems were then inoculated 1:10 into fresh stems, the moisture content adjusted to 1.6 g H₂O/g dry stems, and added to the drums for treatment. Because of the large amount of nitrogen-limited inoculum needed for the initial small column step, the nitrogen-limited inoculum was produced in a slightly different manner than described above. This mycelial inoculum was produced at Utah State University as previously, but the mycelia from the maintenance slants were transferred directly into the nitrogen-limited medium without first being enriched in YM broth. Thus, both of the enrichment steps in the preparation of this mycelial inoculum were carried out in the nitrogen-limited medium. The fungal pellets produced in this manner were harvested as before, shipped under refrigeration to the INEEL, and stored at 4°C until use (up to 4 weeks).

After 6 weeks the treated stems were removed from the glass columns and mixed by hand at 1:10 (w/w) with fresh air-dried uninoculated straw stems. Random samples of the 6-week-degraded stems were dried, ground to 60 mesh, and analyzed for composition as described above. While it was not known how much inoculum would be necessary for the altered inoculation method, a 10 wt% inoculation of wood chips containing an active culture of the desired white-rot fungus has been successful in soil bioremediation (Dr. S.D. Aust, Personal Communication, Utah State University). The moisture content was brought to 1.60 g H₂O/g stems by spraying distilled water with a pressurized garden sprayer onto the fresh stems as they were mixed with the treated straw from the glass columns. The inoculated stems were then packed into 208.5 L (55-gal) drums at about 7.5 kg dry weight of inoculated straw per drum. Before loading the drums, a 56 cm (22 in.) diameter perforated steel disk was placed in the bottom of each drum and elevated to about 5.7 cm (2.25 in.) above the bottom of the drum using screws. Humidified oil-free instrument air at 127.6 kPa (18.5 psia) was supplied at 400 - 500 mL/min to the bottom of each drum beneath the perforated disk; the pressure drop over each drum was about 41.4 kPa (6 psig). The air exited the system separately through the



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centers of the lids of each drum through in-line 16 cm² Whatman HEPA-Vent Filters with a porosity of 0.3 μ m (Whatman, Newton, MA). After 6 or 12 weeks of treatment, the drums were opened and several samples were removed from various locations within the straw beds. The samples were dried, ground to 60 mesh, and analyzed for composition as described above. The drums were then resealed and shipped to the Wood Materials and Engineering Laboratory at Washington State University (WSU) for analyses of various composite formulations and extrusion testing. Untreated straw was also sent to WSU for these analyses. For the composite testing, the straw samples were referred to as "Neat" (untreated), "Degrade1" (treated for 6 weeks), and "Degrade2" (treated for 12 weeks).

Wheat Straw & Fungal Sources & Materials

The composition of the untreated straw stem fraction is shown in Table 9. This composition is an average of four independent samples.

Table 9. Composition of Westbred 936 straw stem fraction used in the fungal treatment studies. Uncertainties given are the standard deviations for 4 independent replicate measurements.

Component	Wt% of Component ^a
Glucan	37.2 \pm 0.8
Xylan	22.1 \pm 0.5
Galactan	1.2 \pm 0.8
Arabinan	3.0 \pm 0.4
Mannan	1.6 \pm 0.4
Lignin with Extractives	18.9 \pm 0.1
Ash	10.1 \pm 0.0
Other ^b	5.8 \pm 2.1
SUM	100.0 \pm 0.2

a. Based on 100% dry weight of material

b. Remaining fraction attributed to unknown uronic acids, proteins, etc., and to recovery errors in analysis techniques.

Nitrogen Consumption Experiments

Since it would be uneconomical to sterilize the straw for use in distributed systems in the existing infrastructure, and also on such a large scale, it is important that the system treat only unsterilized straw. The inoculum preparation tests were done to estimate the initial nitrogen level required to produce a suitable amount of biomass for inoculating the straw while minimizing the amount of nitrogen remaining in the culture medium. These tests were necessary because the slow-growing white rot fungi are late colonizers in nature and express their ligninolytic systems under conditions of nitrogen limitation (Kirk and Farrell 1987). Thus, minimizing the amount of nitrogen that carries over to the straw in the fungal inoculum could allow the inoculated fungi to more quickly overtake the microbes already present in the straw, and thereby minimize or eliminate the need for sterilization of the straw.

Experiments were performed initially using the YM broth/trace minerals medium, with inoculum amounts ranging from 0.1 to 5.0 OD. Tests were run to compare the production of fungal biomass in stationary versus agitated cultures (see Figure 9). As expected, biomass production in the agitated cultures far exceeded that in the stationary cultures, by more than 4 times. The TKN of the cultures was monitored over the course of the experiments, but it did not change significantly even when the cultures entered secondary metabolism (data not shown). This indicated that the cultures were not nitrogen-limited.



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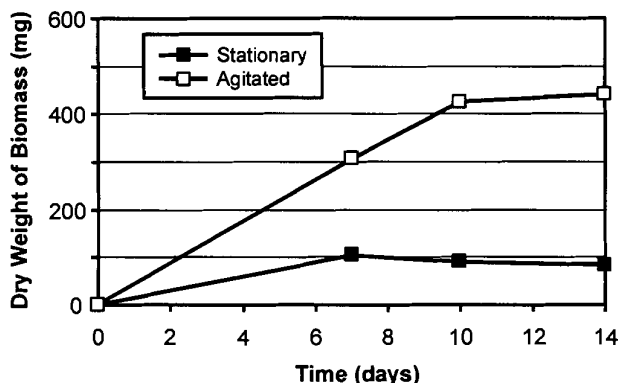


Figure 9. Comparison of *P. ostreatus* biomass production in stationary and agitated cultures. The medium for this test was the YM broth/trace minerals medium.

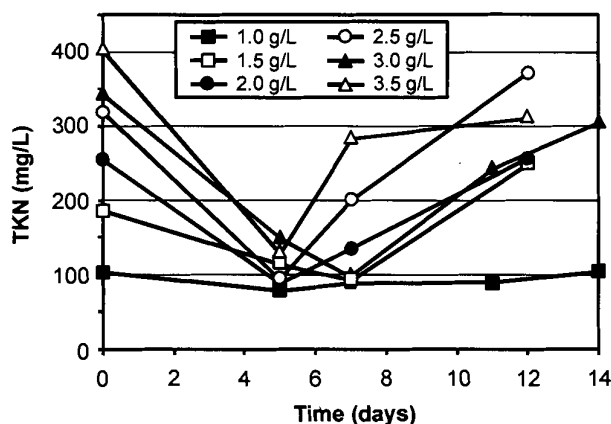


Figure 10. The effect of yeast extract (ca. 10 wt% nitrogen) on the minimum nitrogen level attainable in the inoculum production cultures.

Cultures were then tested in the nitrogen-limited medium, inoculating homogenized mycelia to 1.0 OD. The minimum nitrogen concentration observed in the cultures was about 80 – 100 ppm of TKN (Figure 10). The first sample was taken on day 5 to ensure that a sample would be obtained before the minimum nitrogen concentration was reached (onset of stationary phase), because the original agitated YM broth-grown cultures entered secondary metabolism on or about days 7 – 10, depending on the inoculum amount (not shown). As shown in Figure 10, the minimum nitrogen levels were observed in the first sample on day 5, after which the TKN of the culture fluid increased. The increase in medium nitrogen indicates either exportation of nitrogen from the cells to the medium (as extracellular enzymes), or significant cell death. The shift to earlier onset of stationary phase is consistent with the switch to a more severe nutrient limitation in the new medium. Biomass production did not increase significantly going from 3.0 to 3.5 g/L of yeast extract in the culture.

Preparation of Fungal Inoculum for Straw Upgrading Test Columns

Because biomass production did not increase significantly going from 3.0 to 3.5 g/L of yeast extract in the culture, it was decided to produce inoculum for the column tests using 3.0 g/L of yeast extract in the nitrogen-limited medium. Use of this medium resulted in the production of 5300 – 6700 mg/L of *P. ostreatus* mycelia in 5 days (not shown).

Initial Small-Column Wheat Straw Upgrading Experiments – Determination of Minimum Competitive *P. Ostreatus* Inoculum Size

Proxy Variable for the Initial Upgrading Tests

In these initial degradation tests, a proxy variable – the ratio of cellulose and hemicellulose compositions (C/H) – was used to indicate the relative change in composition occurring from indigenous microbes and by *P. ostreatus*. This ratio was used because *P. ostreatus* has been shown to be somewhat selective for hemicellulose and lignin degradation versus cellulose degradation (Hadar et al. 1993, Lindfelter et al. 1979), while the indigenous microbes were shown in uninoculated controls to be nonselective for one polysaccharide or the other.



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Results of the Initial Upgrading Tests

The initial exploratory experiments were performed to estimate the minimum amount of inoculum necessary to overtake the indigenous microbes in the straw in 12 weeks or less. For these tests, the fungal inoculum amount was varied from 0 – 11 mg of homogenized *P. ostreatus* mycelia per dry gram of air-dried straw stems. The moisture content used for these experiments was 60 – 70% on a dry basis (0.6 – 0.7 g of H₂O/dry gram of stems), based on prior experience at INEEL using compost biofilters to degrade VOCs (Cherry and Thompson, 1997, Kastner et al. 1999). A comparison of the degradation patterns for each inoculum amount is shown in

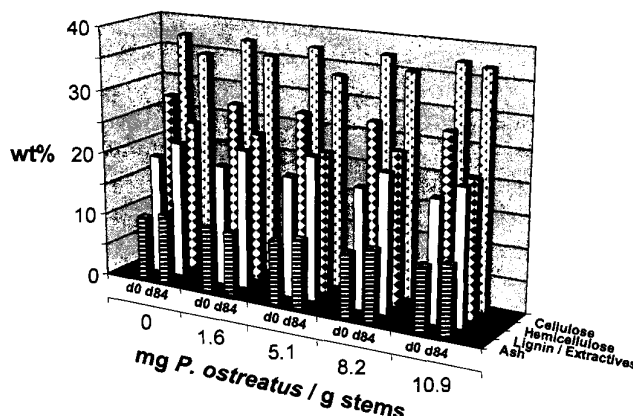


Figure 11. Straw stem compositions at days 0 and 84 as a function of fungal inoculum amounts from 0 – 10.9 mg *P. ostreatus*/g stems.

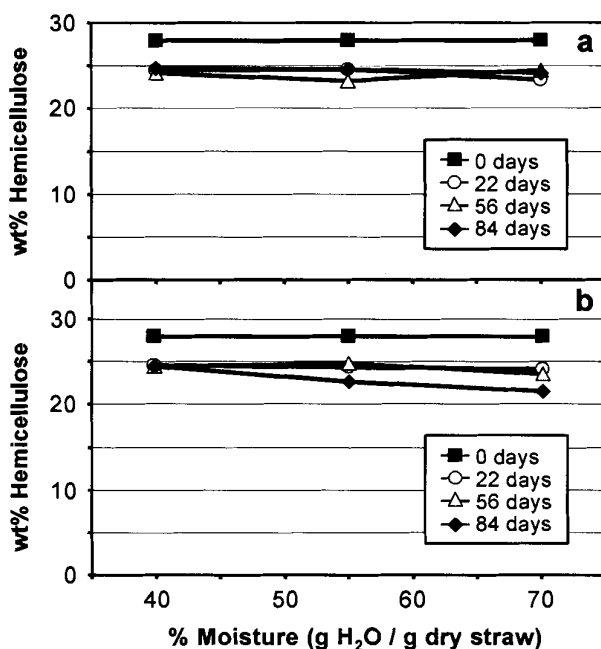


Figure 12. The effect of moisture content on the degradation of hemicellulose at various times. (a) Control (0 mg *P. ostreatus*/g stems); and (b) 10.9 mg *P. ostreatus*/g stems.

Figure 11. Few differences were seen between the control (0 mg *P. ostreatus*/g) and inoculated columns containing less than 5.1 mg/g. Above 5.1 mg/g, relatively less cellulose was degraded with increasing inoculum amount. At 10.9 mg/g, little cellulose was degraded, suggesting dominance of the cultures by *P. ostreatus*. Lignin/extractives contents increased, suggesting lignin was not degraded; however, it is likely that the extractives increased and lignin decreased, as has been shown previously (Moyson and Verachttert 1991).

The next tests performed in the columns were designed to bracket the moisture range necessary for the fungal degradation to proceed. Since hemicellulose is generally the most easily degraded fraction of the stems, hemicellulose degradation was used as the performance measure for these tests. The effect of 40, 55, and 70% moisture on the degradation of hemicellulose at various times over 12 weeks of treatment is shown in Figure 12 for both the uninoculated control and for the 10.9 mg/g columns. Moisture had

little effect on straw stem degradation in control columns below 70% (0.7 g H₂O/g dry stems) (see Figure 12a). In columns inoculated with *P. ostreatus*, there was a slight effect of moisture below 70% (Figure 12b), but only after 60 days of culture. Although this effect was not significant at 55% moisture, there was a linear trend of increased hemicellulose removal at 84 days going from 40 to



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70% moisture (Figure 12b). In all experiments, including those using higher than 5.1 mg/g inoculum, the first 3 weeks of degradation gave nearly identical results, indicating that even though dominance by *P. ostreatus* was observed at 84 days using higher inoculum amounts, the initial 3 weeks of the culture were still dominated by the indigenous organisms. This may be due to nitrogen levels being too high during this period, or to a significant lag time in *P. ostreatus* growth during its acclimation to straw stems as a substrate for growth. In any event, it seems clear that higher moisture contents will give increased degradation of hemicellulose in the column tests; hence, we began testing moisture contents from 90 – 150% (dry basis).

Determination of Dominance of Inoculated Fungus

The last set of initial column tests was designed to determine the amount of inoculum necessary to obtain suitable feedstock for production of straw thermoplastic composites, while obtaining this composition in 12 weeks or less. It is expected that removal of hemicellulose will allow better resin penetration and adhesion, since similar results have been obtained using steam exploded broom fibers (Avella et al. 1998). The fiber damage imparted by steam explosion would not be expected to be present in straw stems treated with fungi, especially by a fungus that does not degrade significant cellulose (Hadar et al. 1993). However, for this to occur the inoculated *P. ostreatus* must overtake the indigenous microbes. Since *P. ostreatus* is selective for hemicellulose and lignin degradation versus cellulose degradation, a clear signal that the inoculated *P. ostreatus* has overtaken the indigenous organisms would be a significant increase in the cellulose/hemicellulose ratio. Therefore, the laboratory performance measures for these experiments were set as increased removal of hemicellulose, together with an increased ratio of cellulose/hemicellulose. The composite material properties will dictate the final inoculum amount and treatment time chosen.

The cellulose/hemicellulose ratios and percent hemicellulose removal data at zero and 84 days for the 70% moisture column treatments are shown in Table 10. In only the 10.9 mg/g case was the cellulose/hemicellulose ratio increased significantly. This indicates that the *P. ostreatus* inoculum amount should be set at 10.9 mg/g or greater. Thus, the inoculum amount range for these experiments will be 11 – 100 mg/g or higher. Nearly 60% removal of hemicellulose in 12 weeks has been shown in the literature using *Pleurotus* species (Moyson and Verachtert 1991). At 10.9 mg/g inoculum, we achieved a nearly 25% reduction in hemicellulose with little loss of cellulose. Thus, increased inoculum amounts over 10.9 mg/g are expected to increase the amount of degradation in the same time period.

Table 10. Cellulose/hemicellulose ratios at zero and 84 days for the 70% moisture column treatments. A significantly increased cellulose/hemicellulose ratio versus the day zero ratio indicates selective degradation of hemicellulose versus cellulose, and thus degradation by *P. ostreatus* that is relatively greater than that by indigenous organisms.

Inoculum Amount (mg <i>P. ostreatus</i> /g stems)	Cellulose/ Hemicellulose	Organism(s) Assumed Dominant	% Removal of Hemicellulose in 84 days
Day 0	1.33 ± 0.08	---	---
0	1.43 ± 0.05	Indigenous	13.7
1.6	1.49 ± 0.05	Indigenous	15.1
5.1	1.53 ± 0.09	Indigenous	21.7
8.2	1.46 ± 0.09	Indigenous	14.1
10.9	1.75 ± 0.11	<i>P. ostreatus</i>	24.8



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Subsequent Small-Column Wheat Straw Upgrading Experiments – Determination of Variations in Final Straw Compositions with Moisture Content and Inoculum Size

Proxy Variable for the Subsequent Upgrading Tests

Due in part to the low, and thus more uncertain, measurements of the non-xylan carbohydrate components of hemicellulose (galactan, arabinan and mannan), it was decided to change the proxy variable to one exhibiting less variability due to measurement uncertainty. Two additional proxy ratios were assessed, including the ratio of glucan and xylan compositions (G/X) and an estimate of the relative degradation of xylan versus glucan ($\Delta X/\Delta G$). This "degradation ratio" was calculated from the estimated conversions, which assumed little change in the sum of ash, lignin, and extractives. Implicit in this is an assumption of minimal mineralization of lignin to CO_2 , and thus losses of lignin are assumed to be from depolymerization to extractives; extractives would increase, keeping the sum of lignin and extractives constant. This allowed a closed mass balance to be estimated and the amounts of xylan and glucan degraded to be calculated on an initial weight basis.

A comparison of these ratios for the preliminary testing at 0 – 10.9 mg *P. ostreatus*/g stems and 0.77 g H_2O /g stems is shown in Table 11. The majority of the glucan/xylan-based ratios had lower standard deviations than the corresponding cellulose/hemicellulose-based ratios, which was expected because xylan represents a greater fraction of the straw stems than galactan, arabinan, and mannan (Table 9). The relative changes in the three proxy variables were consistent among the data. For example, when C/H and G/X did not change significantly, indicating nonselective degradation of glucan and xylan, the estimated degradation ratio $\Delta X/\Delta G$ was about 1. Similarly, when C/H and G/X exhibited only a small increase, $\Delta X/\Delta G$ was only slightly larger than 1, while larger increases in C/H and G/X corresponded with large increases in $\Delta X/\Delta G$. This may provide some support for the assumption used to estimate $\Delta X/\Delta G$. The proxy ratio chosen for use was the degradation ratio $\Delta X/\Delta G$; with this change, the conclusions of the preliminary study were unchanged, i.e. *P. ostreatus* was shown to out-compete the indigenous organisms by 56 days. In addition, from 0 – 22 days the $\Delta X/\Delta G$ ratios did not change significantly from 1.0, suggesting that under the conditions tested, in the initial 22 days of culture *P. ostreatus* did not dominate degradation of the stems.

Effect of Process Variables on Xylan and Glucan Removal and Selectivity

The time courses of the polysaccharide, lignin with extractives, and ash contents for experiments conducted at 44.0 mg *P. ostreatus*/g stems and 1.60 g H_2O /g stems are shown in Figure 13. Time courses such as these were used to estimate xylan and glucan conversions for separate replicate samples, which were then averaged. The xylan and glucan conversions and $\Delta X/\Delta G$ ratios for 23.0 – 149 mg *P. ostreatus*/g stems and 1.10 – 2.24 g H_2O /g stems are shown in Table 12. Refer to Figure 8 for the complete set of inoculum and moisture combinations tested. Of the moisture and inoculum combinations shown in Figure 8, the (moisture, inoculum) combinations (0.77, 21.0), (0.90, 34.0), and (1.20, 41.0) were performed early in the study and displayed visually uneven growth of fungus in the stems, indicating inhomogeneous distribution of the mycelia over the straw stem surface. Samples taken from the tops and bottoms of these columns also displayed widely variable degradation results, indicating poor distribution of the inoculum. The methods were modified and the uneven distribution of fungal mycelia on the straw was minimized in future experiments. These three data sets were thus not considered further in analyses of the data. The $\Delta X/\Delta G$ ratios for the preliminary tests conducted at 0.0 – 10.9 mg *P. ostreatus*/g stems and 0.40 – 0.77 g H_2O /g stems are given in Table 11.



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Table 11. Comparison of cellulose/hemicellulose, glucan/xylan, and xylan degradation/glucan degradation ratios for the initial upgrading tests at 0.77 g H₂O/g dry stems. Uncertainties given are the standard deviations for 12 independent replicate measurements.

Day / Ratio	Inoculum Amount (mg <i>P. ostreatus</i> / g dry stems)				
	0	1.6	5.1	8.2	10.9
Day 0					
C/H ^a	1.33 ± 0.08	1.33 ± 0.08	1.33 ± 0.08	1.33 ± 0.08	1.33 ± 0.08
G/X ^b	1.69 ± 0.06	1.69 ± 0.06	1.69 ± 0.06	1.69 ± 0.06	1.69 ± 0.06
ΔX/ΔG ^c	NA ^d	NA	NA	NA	NA
Day 22					
C/H	1.47 ± 0.04	1.45 ± 0.04	1.44 ± 0.05	1.37 ± 0.08	1.43 ± 0.05
G/X	1.71 ± 0.06	1.72 ± 0.06	1.69 ± 0.06	1.72 ± 0.06	1.68 ± 0.05
ΔX/ΔG	1.11 ± 0.14	1.11 ± 0.07	1.02 ± 0.06	1.14 ± 0.04	0.95 ± 0.08
Day 56					
C/H	1.36 ± 0.03	1.43 ± 0.06	1.52 ± 0.04	1.55 ± 0.06	1.50 ± 0.07
G/X	1.64 ± 0.03	1.73 ± 0.04	1.80 ± 0.12	1.84 ± 0.13	1.87 ± 0.07
ΔX/ΔG	0.85 ± 0.11	1.19 ± 0.04	1.26 ± 0.07	1.18 ± 0.08	1.78 ± 0.42
Day 84					
C/H	1.43 ± 0.05	1.49 ± 0.05	1.53 ± 0.09	1.46 ± 0.09	1.75 ± 0.11
G/X	1.70 ± 0.02	1.73 ± 0.01	1.77 ± 0.05	1.73 ± 0.03	1.82 ± 0.07
ΔX/ΔG	1.04 ± 0.02	1.15 ± 0.05	1.17 ± 0.13	1.12 ± 0.03	1.52 ± 0.10

a. Cellulose/Hemicellulose composition ratio

b. Glucan/Xylan composition ratio

c. Xylan/Glucan degradation ratio. This ratio was calculated assuming little change in the sum of ash, lignin, and extractives.

d. NA = Not applicable since on day 0 no degradation has occurred

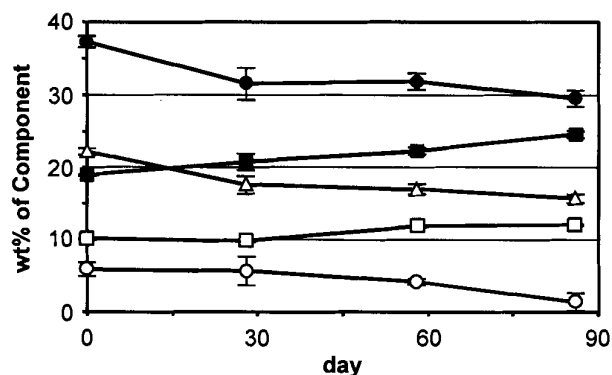


Figure 13. Time courses of straw stem components for upgrading of stems using 44 mg *P. ostreatus*/g stems at a moisture content of 1.6 g H₂O/g stems. Symbols are: (●) glucan; (Δ) xylan; (■) lignin with extractives; (□) ash; and (○) the sum of galactan, arabinan, and mannan.



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Table 12. Xylan and glucan conversions for the upgrading of wheat straw stems using *P. ostreatus* at 23.0 – 149 mg/g stems and moisture contents of 1.10 – 2.24 g H₂O/g stems. Uncertainties given are the standard deviations for 12 independent replicate measurements.

Inoculum (mg <i>P. ostreatus</i> / g stems)	Moisture (g H ₂ O/g stems)	ΔX (%)	ΔG (%)	$\Delta X/\Delta G$
4 weeks				
23.0	1.77	23.4 ± 3.0	16.4 ± 3.2	1.44 ± 0.09
44.0	1.60	24.3 ± 7.6	19.2 ± 8.4	1.31 ± 0.18
70.0	1.10	19.6 ± 1.8	15.3 ± 1.6	1.28 ± 0.01
92.0	1.20	25.2 ± 4.0	18.2 ± 4.2	1.40 ± 0.12
105	2.24	26.4 ± 1.0	19.0 ± 0.6	1.39 ± 0.01
149	1.64	22.9 ± 0.2	17.9 ± 1.0	1.28 ± 0.06
8 weeks				
23.0	1.77	29.7 ± 0.9	21.8 ± 3.1	1.38 ± 0.15
44.0	1.60	34.5 ± 3.5	27.1 ± 3.7	1.28 ± 0.05
70.0	1.10	35.4 ± 0.9	26.6 ± 1.3	1.33 ± 0.05
92.0	1.20	32.3 ± 3.2	25.8 ± 3.1	1.25 ± 0.03
105	2.24	38.8 ± 0.5	32.2 ± 0.5	1.21 ± 0.01
149	1.64	39.7 ± 0.5	33.6 ± 3.0	1.19 ± 0.09
12 weeks				
23.0	1.77	36.3 ± 1.7	29.3 ± 2.7	1.25 ± 0.06
44.0	1.60	43.8 ± 2.6	37.3 ± 3.1	1.18 ± 0.03
70.0	1.10	37.0 ± 2.7	32.5 ± 3.6	1.14 ± 0.04
92.0	1.20	46.7 ± 3.1	38.3 ± 3.0	1.22 ± 0.04
105	2.24	48.3 ± 4.3	41.2 ± 5.4	1.18 ± 0.05
149	1.64	42.9 ± 2.6	37.9 ± 2.2	1.13 ± 0.05

Effect of Treatment Time

Treatment time is an important process variable because large process footprints such as those required for this type of treatment require large amounts of land, and thus long treatment times can have a negative influence of the economics of the process, depending on land use requirements (Hatakka 1983). In addition, depending on the sensitivity of the treatment process to initial and transient conditions, widely variable product compositions produced in time-sensitive degradation processes could have a large effect on the next step of the manufacturing process. Longer treatments gave progressively smaller gains in xylan removal and larger gains in glucan removal (Table 12). This observation is consistent with the fungus first utilizing the easily-degraded hemicellulose and amorphous cellulose fractions, beginning with the hemicellulose.

In 4 weeks, the maximum observed conversions were about 27% xylan and 19% glucan. At 8 weeks, the maximum conversions observed were about 40% xylan and 34% glucan, while at 12 weeks maximum conversions of about 48% xylan and 41% glucan were observed. At very low inoculum levels, the initial degradation (at 4 weeks) was generally nonselective and thus primarily due to indigenous organisms (Table 11). Above 10.9 mg *P. ostreatus*/g stems, the early degradation was much more selective relative to the indigenous organisms, indicating significant activity of the inoculated fungus. Maximum selectivities for xylan removal ($\Delta X/\Delta G$) for inoculum levels exceeding 10.9 mg *P. ostreatus*/g stems were observed earlier in the



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treatments, with all moisture and inoculum combinations showing similar selectivities after 12 weeks of treatment.

Effects of Moisture and Inoculum

Higher conversions of both xylan and glucan were seen with increases in both moisture content and inoculum size (Table 12), but no correlation was observed between the conversions and the relative amounts of inoculum and moisture (ratio of inoculum to moisture content; not shown). Thus, it is unlikely that these two parameters comprise an interaction effect that is important to the operation of the system. Lower moisture contents gave lower overall amounts of degradation, but seemingly better selectivities for xylan degradation although coefficients of variation for conversions were higher at low moisture contents due to the smaller changes in overall composition. Higher moisture gave better overall degradation but poorer selectivity for xylan degradation. Selective xylan degradation may not have as great an effect on the properties of straw-thermoplastic composites as may overall degradation. In this study, selectivity for xylan removal was a convenient proxy measure of relative activity of the inoculated fungus to indigenous microbes. However, there are other uses for treated straw feedstock, such as for production of fermentable sugars for fuels and chemicals, in which selective xylan removal would be useful. If achieving high selectivity for xylan degradation is important to the final use of the feedstock, lower moisture levels would be preferred. Finally, higher inoculum was found to give faster overall degradation, which was expected.

Regression and Sensitivity Analyses of the Degradation Data

Although the tests were performed in an exploratory manner and thus neither a complete factorial design nor a complete fraction of a factorial design was completed, a significant amount of revealing information was collected in the tests. Since the goal was to bracket allowable moisture and inoculum ranges, statistical analyses of the xylan and glucan degradation data were conducted by regression analysis and used to explore system sensitivity to initial moisture and inoculum contents.

The conversion data for all tests were combined into a single data set represented by 234 data points varying in inoculum amount (I , mg *P. ostreatus*/g stems), gravimetric moisture content (M , g H_2O /g stems), and treatment time (t , days). A power series expansion of the three variables through the second-order terms was fitted using linear regression; the expansion included the terms I , M , t , IM , It , Mt , I^2 , M^2 , and t^2 , with an intercept of zero. Note that this equation has no basis in theory and was chosen simply because its shape was appropriate. The primary goal of the regression analyses was to obtain statistically valid equations for both ΔX and ΔG , and to use these relationships to estimate the sensitivity of the system to inoculum, moisture, and treatment time.

The xylan conversion (ΔX) and glucan conversion (ΔG) data were fitted separately to the power series expansion, resulting in r^2 values of 0.925 and 0.910, respectively. However, an analysis of variance indicated that the terms M , IM , M^2 in both analyses were statistically insignificant and thus unnecessary to fit the data. The data were re-fitted after dropping those terms, resulting in statistically valid fits with r^2 values of 0.924 and 0.909. The results of the regression analyses are presented for both fits in Table 13, and comparisons of the measured and predicted values of ΔX and ΔG are shown in Figures 14 and 15, respectively. Relatively good fits to the data were obtained, indicating that the data were internally consistent and that the system behaved in a predictable manner. The fits were more accurate at higher values of inoculum and moisture, caused by the higher amount of variability in degradation data at lower



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Table 13. Regression models for the xylan and glucan conversions.

	Regression Results ^a	
	$\Delta X = \alpha_x I + \beta_x t + \gamma_x I t + \delta_x M t + \epsilon_x I^2 + \phi_x t^2$	$\Delta G = \alpha_g I + \beta_g t + \gamma_g I t + \delta_g M t + \epsilon_g I^2 + \phi_g t^2$
DOF ^b	233	233
r ²	0.924	0.909
α_i	0.106	0.0551
β_i	0.514	0.408
γ_i	1.68×10^{-3}	1.43×10^{-3}
δ_i	0.106	0.0770
ϵ_i	-8.19×10^{-4}	-4.45×10^{-4}
ϕ_i	-4.11×10^{-3}	-2.89×10^{-3}

a. Variable definitions: ΔX (xylan conversion, %); ΔG (glucan conversion, %); I (inoculum amount, mg *P. ostreatus*/g stems); M (moisture content, g H₂O/g stems); t (time, days).

b. DOF = Degrees of freedom for the regression analysis.

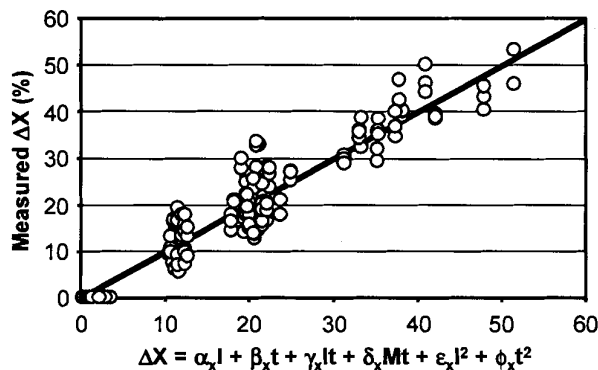


Figure 14: Comparison of the predicted and measured xylan conversions for the fungal upgrading tests. The line shown has a slope of 1.0 and represents a perfect fit to the data.

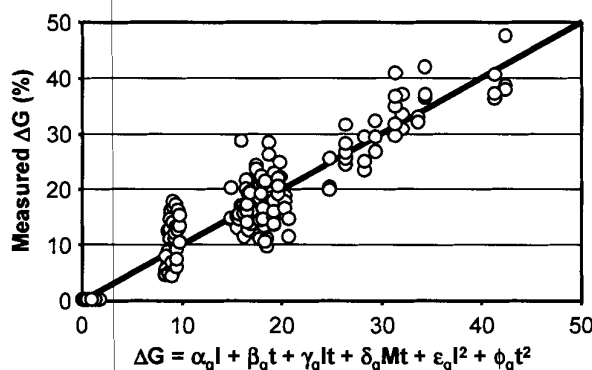


Figure 15: Comparison of the predicted and measured glucan conversions for the fungal upgrading tests. The line shown has a slope of 1.0 and represents a perfect fit to the data.

inoculum levels and moisture contents. The predicted conversions of xylan (ΔX), glucan (ΔG), and the ratio $\Delta X/\Delta G$ with time are shown for the ultimately-selected treatment conditions (40 mg *P. ostreatus*/g stems, 1.60 g H₂O/g stems) in Figure 16. The percentages of degradation for the nearest experimentally observed combination (44.0 mg *P. ostreatus*/g stems, 1.60 g H₂O/g stems) were under-predicted by 5 – 10% at later treatment times (not shown). It is clear from Figure 16 that the time-rate of degradation has decreased substantially by 12 weeks and thus harvesting at 10 or 14 weeks would make little difference in the final composition. In addition, the selectivity for xylan degradation over glucan degradation is predicted to be initially about 2.0 and then to decrease with time to about 1.2. This suggests that shorter treatment times would be preferred with this organism if a more selective degradation is desired, although the initially high rate of decline of $\Delta X/\Delta G$ is most likely an artifact of higher measurement uncertainties in the data at lower moisture and inoculum.



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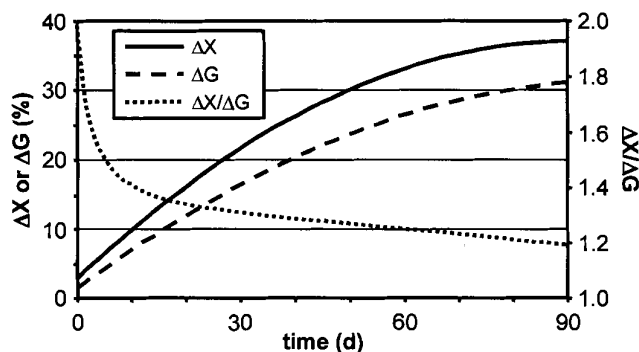


Figure 16. Predicted time courses of xylan conversion (ΔX , %), glucan conversion (ΔG , %), and the degradation ratio ($\Delta X/\Delta G$) for 40 mg *P. ostreatus*/g stems and 1.60 g H_2O /g stems.

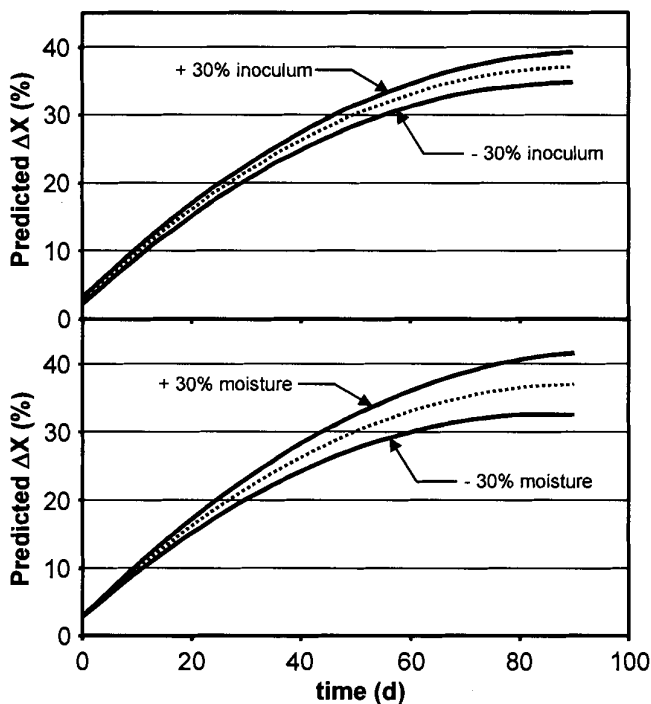


Figure 17. Inoculum and moisture sensitivity analyses for fungal treatment of straw stems at $21 \pm 2^\circ C$. Top panel: $\pm 30\%$ variation of inoculum at 1.6 g H_2O /g stems. The dotted line is the prediction for the midpoint of 40 mg *P. ostreatus*/g stems. Bottom panel: $\pm 30\%$ variation of gravimetric moisture content at 40 mg *P. ostreatus*/g stems. The dotted line is the prediction for the midpoint of 1.6 g H_2O /g stems.

Sensitivity analyses were conducted using the regression models to test the sensitivity of the treatment method at a temperature of $21 \pm 2^\circ C$. First, the inoculum was varied $\pm 30\%$ in the regression model (28.0 – 52.0 mg *P. ostreatus*/g stems) at constant moisture. Next, the moisture was separately varied $\pm 30\%$ (1.12 – 2.08 g H_2O /g stems) at constant inoculum. The upper and lower bounds chosen represent very large variations in both inoculum and moisture. The predictions are plotted versus time in Figure 17 for xylan degradation. The xylan degradation ranges at 12 weeks for varied inoculum and moisture were 34.7 – 39.3% and 32.5 – 41.6% degraded, respectively. Similarly, the glucan degradation ranges at 12 weeks for varied inoculum and moisture were 29.2 – 32.8% and 27.7 – 34.4% degraded, respectively (not shown). When varying only one parameter, the final compositions are predicted to be relatively insensitive to inoculum size, with the largest deviation of at most $\pm 5\%$ degradation at 12 weeks. For moisture the system was predicted to be more sensitive, but it was less sensitive at shorter degradation times. This indicates that initial moisture is the more critical parameter to control, and also that the system is less sensitive to initial moisture at shorter treatment times. Shorter treatment times could be used without compromising final compositions by increasing initial inoculum size, depending on final costs.

Additional sensitivity analyses were conducted by simultaneously varying both inoculum amount and moisture content ($\pm 30\%$ for each). This analysis predicted maximum ranges at 6 and 12 weeks of 24.5 – 31.9% and 30.1 – 43.8% xylan degraded, and 19.4 – 24.7% and 25.9 – 36.1% glucan degraded, respectively. This corresponds to $\Delta X/\Delta G$ ranges of 1.27 – 1.29 and 1.16 – 1.21 at 6 and



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12 weeks, respectively, and indicates that the expected $\Delta X/\Delta G$ does not vary as widely as would be suggested by combining the individual sensitivity analyses. In addition, shorter treatment times were again favored for minimum variation in the treated straw stem compositions.

In terms of selectivity and reduced sensitivity to initial moisture and inoculum, the results indicate that shorter treatment times are preferred, especially if moisture is either not controlled or is poorly controlled. The regression models were next used to generate topographical plots of ΔX , ΔG , and $\Delta X/\Delta G$ at the various combinations of inoculum and moisture. The results are plotted in Figures 18 – 20 for the regression model predictions after 6 weeks of treatment. For locations of the parameter combinations used in this study on these plots, refer to Figure 8 (which uses the same axes). Note that the parameter combinations (21.0, 0.77), (34.0, 0.90), and (41.0, 1.20) were not included in the statistical analyses because of poor distribution of the fungal inoculum onto the straw stems. The topographical plot of percentage xylan degraded after 6 weeks of treatment is shown in Figure 18. The closed diamond represents the conditions chosen for preparation of treated stems for the extrusion testing. The region of only 15 – 20% xylan degradation roughly corresponds to the region in which the inoculated *P. ostreatus* was observed to be unable to outcompete the indigenous microbes, as about 15% xylan degradation was observed to occur without inoculum. Increased xylan removal is predicted as both moisture and inoculum increase. There are, however, wide ranges of parameter combinations that will give the same amount of xylan degradation, indicating a fairly insensitive system in terms of overall xylan degradation after 6 weeks of treatment. The curvature of the dividing curve between 25 – 30% and 30 – 35% xylan degradation, and above 100 mg *P. ostreatus*/g stems, seems odd in that it curves back toward the inoculum axis. However, this was experimentally observed by comparing the results of the (149, 1.67) and (105, 2.24) parameter combinations (see Figure 8). Since these experiments were independently replicated, the behavior appears to be real.

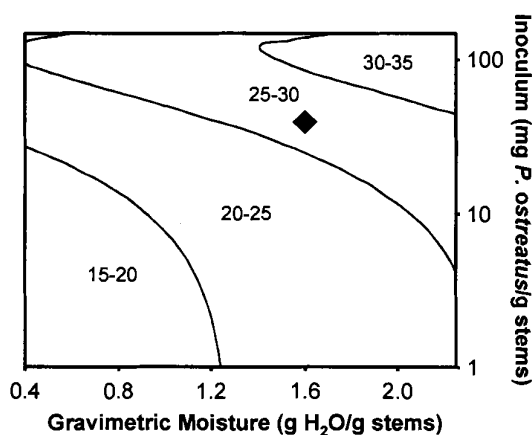


Figure 18. Predicted topographical plot of xylan conversion (ΔX , %) with inoculum amount and moisture content. The ranges presented on the plot are ranges of xylan conversion predicted in each region. The closed diamond marks the conditions chosen for preparation of treated stems for the extrusion tests.

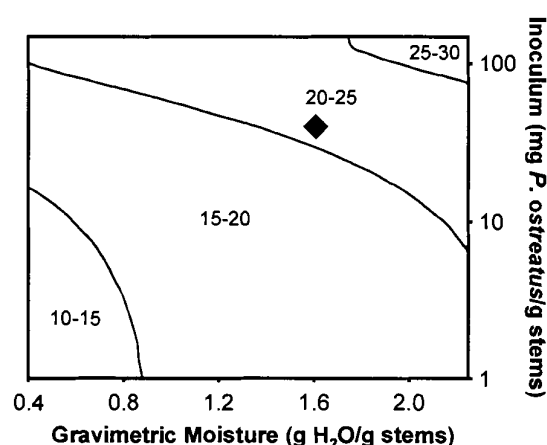


Figure 19. Predicted topographical plot of glucan conversion (ΔG , %) with inoculum amount and moisture content. The ranges presented on the plot are ranges of glucan conversion predicted in each region. The closed diamond marks the conditions chosen for preparation of treated stems for the extrusion tests.



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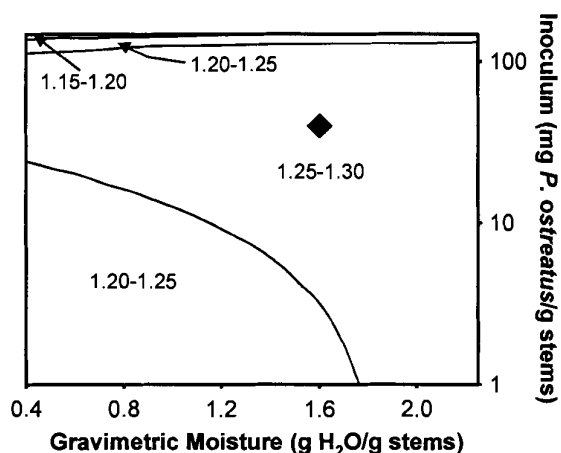


Figure 20. Predicted topographical plot of the degradation ratio ($\Delta X/\Delta G$) with inoculum amount and moisture content. The ranges presented on the plot are ranges of $\Delta X/\Delta G$ predicted in each region. The closed diamond marks the conditions chosen for preparation of treated stems for the extrusion tests.

The topographical plot of percentage glucan degraded after 6 weeks of treatment is shown in Figure 19. Again, the closed diamond represents the conditions chosen for preparation of treated stems for the extrusion testing. The region of only 10 – 15% glucan degradation closely corresponds to the experimentally-observed region in which the inoculated *P. ostreatus* was unable to outcompete the indigenous microbes. Increased glucan removal is predicted as both moisture and inoculum increase. As shown above for xylan, there are again wide ranges of parameter combinations that give the same amount of glucan degradation, indicating that the system is also fairly insensitive in terms of overall glucan degradation after 6 weeks of treatment.

Finally, the topographical plot of $\Delta X/\Delta G$ after 6 weeks of treatment is shown in Figure 20. The closed diamond again represents the conditions chosen for preparation of treated stems for the extrusion testing. The region of $\Delta X/\Delta G$ of 1.20 – 1.25 encompasses the region

in which it was experimentally observed that $\Delta X/\Delta G$ was about 1.0. A ratio of $\Delta X/\Delta G$ of 1.0 indicates non-selective polysaccharide degradation and was taken as indication of poor competition of the inoculated fungus with the indigenous microbes. This reinforces the observations that at low moisture and inoculum, the regression model predictions are less accurate. Figure 20 also shows that $\Delta X/\Delta G$ of 1.25 – 1.30 is predicted after 6 weeks of treatment over a very large percentage of the possible moisture and inoculum combinations. Thus, the system is very stable with respect to selectivity of polysaccharide degradation within the parameter ranges tested.

Larger-Scale Laboratory Treatment in Drums

The larger-scale columns, while necessary to produce treated straw stems for use in the preparation of composite formulations for extrusion testing, were also a good test of the sensitivity of the system to scale-up to larger columns and to changes in inoculum source and inoculation method. After 6 weeks of treatment in the small columns used to inoculate the drums, 28 – 30% xylan was degraded while about 18.5% of the glucan was degraded (Table 14). This gave $\Delta X/\Delta G$ ratios of 1.52 – 1.64. For comparison, at 40.0 mg *P. ostreatus* /g stems and 1.60 g H_2O /g stems, the regression models predict 27.2% xylan degradation and 21.1% glucan degradation at 6 weeks, for a $\Delta X/\Delta G$ of 1.29. Clearly, *P. ostreatus* was dominant in the small columns used to prepare enrichment inoculum for the barrels. However, the $\Delta X/\Delta G$ values were well outside the range predicted by the regression models. Apparently, the nitrogen-limited inoculum produced by Utah State University and shipped to INEEL for these columns was either more active or was better acclimated to the nitrogen-limited conditions in the straw stems. This effect was repeatable, indicating that the history of the inoculum used may have a significant effect on $\Delta X/\Delta G$, although the actual glucan and xylan conversions were not far from the predicted values. The inoculum produced for the small columns used to inoculate the drums was produced differently than the inoculum used to inoculate the small-columns in the moisture and inoculum tests—it was better acclimated to nitrogen-limited conditions. This is



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Table 14. Xylan and glucan conversions and degradation ratios estimated for upgrading of wheat straw stems using *P. ostreatus* at 40.0 mg/g stems and moisture content of 1.60 g H₂O/g stems in the scaled-up columns. Uncertainties given are the standard deviations for 8 independent replicate measurements.

Component degraded	Percentage degraded after 6 weeks of treatment			
	Degrade1		Degrade2	
	Small Columns ^a	55-gal Drums	Small Columns ^a	55-gal Drums
ΔX (%)	29.8 \pm 1.0	11.9 \pm 0.9	28.1 \pm 1.0	24.2 \pm 4.9
ΔG (%)	18.3 \pm 1.4	13.4 \pm 1.5	18.5 \pm 1.2	26.8 \pm 3.4
$\Delta X/\Delta G^a$	1.64 \pm 0.09	0.89 \pm 0.03	1.52 \pm 0.05	0.90 \pm 0.08

a. Columns were inoculated at the indicated concentrations of *P. ostreatus* and moisture concentration and grown for a 6 week time period then this inoculum was used to inoculate drums at a 1:10 weight ratio.

because the mycelia were transferred directly into the nitrogen-limited medium (C/N of 32.6) without first being grown in the carbon-limited YM broth (C/N of 7.7). Since both enrichment steps in the production of the mycelial inoculum were carried out in the nitrogen-limited medium, this likely resulted in a mycelial inoculum that was better acclimated to low-nitrogen conditions when it was added to the straw stems, which have a C/N of about 80 (Steinegger and Janssen 1993). Thus, system performance is sensitive to inoculum source and history.

The altered inoculation method also resulted in a different degradation pattern than that observed in the small-column tests. After 6 and 12 weeks of treatment in the drums, only 11.9 and 24.2% of the xylan was degraded, respectively (Table 14). Glucan degradation was similarly reduced, with only 13.4 and 26.8% of the glucan degraded at 6 and 12 weeks, respectively. This equates to $\Delta X/\Delta G$ values of 0.89 and 0.90 at 6 and 12 weeks, respectively. Thus, selective degradation did not occur in the larger-scale columns which indicates that *P. ostreatus* was not dominant. In fact, less degradation occurred in the barrels after 6 weeks of degradation than was either observed or predicted in the small columns. It is likely that the low levels of degradation observed in the drums were due to slower colonization of the fresh stems by the *P. ostreatus* growing in the solid enrichment inoculum. In the small-column tests, the indigenous microbes were shown to degrade about 15 – 20% of the polysaccharides in 12 weeks in the absence of *P. ostreatus*, which likely represents the most easily accessible glucan and xylan fractions. If *P. ostreatus* were to colonize the straw more slowly from the solid enrichment inoculum, the primary effect on the degradation system would be to extend the treatment time necessary to reach the desired levels of xylan and/or glucan degradation in the final product. Thus, inoculating the fresh stems with partially-degraded stems before introduction to the drums was an ineffective inoculation method when compared to inoculating by spraying homogenized mycelia onto the stem surfaces. The nonselective degradation pattern in the drums may or may not be a detriment to the physical properties of straw-thermoplastic composites produced from Degrade1 and Degrade2 straw stems, since selectively-degraded stems have not been compared with nonselectively-degraded stems.



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Task 2b – Fungal Preprocessing of Straw Residue, Pilot-Scale Fungal Inoculation and Degradation of Wheat Straw

Investigators: Anke Schirp, Frank J. Loge, Michael P. Wolcott and Karl Englund

Performing Institution: Washington State University

In this task, we explore the feasibility of production and utilization of fungal-modified wheat straw as filler in high-density polyethylene-based composites. We present observations relating to the mechanical, physical, chemical and thermal properties of fungal-modified straw and the derived straw-polyethylene composites, and we explore the beneficial effects of initial straw sterilization for optimizing growth of the lignin-degrading fungus *Pleurotus ostreatus* (Jacq. ex Fr. Kummer).

The specific objectives of this study were to:

1. Characterize physical, chemical, thermal and morphological properties of wheat straw following treatment with *P. ostreatus*; and
2. Evaluate the influence of straw sterilization on the effectiveness of straw degradation by *P. ostreatus*.

Wheat Straw and Preparation of Fungal Inoculum

A hard red spring variety of wheat straw (*Triticum aestivum* var. Westbred 936), obtained from Grant 4-D Farms (Rupert, ID) during the year 2000 cropping season, was used in all experiments. Straw stems were mechanically separated and stored indoors at $21 \pm 2^\circ\text{C}$ and 13% moisture content until used (Houghton et al. 2004). Overall straw stem length was less than ~ 10 cm with typical values ranging from 5 and 10 cm. Some of the straw used in the experiments was chopped through a screen with a 1.78 cm-hole-diameter using a Nelmor-chopper.

Pleurotus ostreatus ATCC 32783 was chosen based on its lignin-degrading activity (Haider and Trojanowski 1975). Fungal stock cultures were maintained at Utah State University on agar slants containing 41 g yeast-malt (YM) agar (Becton, Dickinson and Company, Sparks, MD) per one liter of water. Fresh slants were prepared and inoculated every two weeks. Slants were incubated at room temperature with caps loosely attached. Fungal inoculum from two- to three-week old slants were used to inoculate 100 ml of liquid starter cultures in 500 ml Erlenmeyer flasks containing 21 g YM broth (Becton, Dickinson and Company, Sparks, MD) per one liter of water and 10 ml of a mineral solution, consisting of 0.03 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 5 mg of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.01 g of NaCl, 1 mg of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1 mg of CoSO_4 , 1 mg of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1 mg of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 mg of $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$, 0.1 mg of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.1 mg of $\text{Al}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$, 0.1 mg of H_3BO_3 (all chemicals were standard laboratory grade). The mineral solution was dissolved in 1.5 g per liter of nitrilotriacetic acid, with the pH adjusted to 6.5 with KOH, and added to the autoclaved YM broth using filter sterilization. Starter cultures were incubated for two to three days at room temperature in a metabolic shaker (New Brunswick Scientific Series 25, Edison, NJ) at ~200 rpm. After sufficient growth, the starter cultures were aseptically transferred to 2.8 liter Fernbach flasks containing 1.5 liter of liquid culture media (same as for starter cultures) and incubated as previously for three to four days. Fungal pellets were separated from medium using a centrifuge (Sorvall RC-5B) at ~10,000g for 10 – 15 min and transferred to sterile 500 ml bottles with sufficient spent medium to submerge the pellets. These bottles were shipped under refrigeration to Washington State University and stored at 4°C until use (no longer than two weeks from date of arrival).



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Affect of Fungal Degradation on Wheat Straw – Mushroom Spawn Bag Tests

Wheat straw was treated with different amounts of fungal inoculum and sterile, distilled water (Table 9). For each treatment, except for #12, 100 g of wheat straw were placed in each of three 53.3 x 21.0 x 12.1 cm mushroom spawn bags (Mycos Supply, Pittsburgh, PA), equipped with a 0.3 μ m filter patch which allowed gas exchange while simultaneously precluding the passage of contaminants during incubation. Inoculation was performed as follows: 500 ml of fungal inoculum in spent medium were transferred into a sterile blender (Waring, Torrington, CT), and 50 ml sterile, distilled water were added to aid dissolution. The solution was homogenized in the blender for 30 seconds at low speed setting. Sixty ml of sterile, distilled water were added to each bag to obtain a straw moisture content (MC) of ~70% (based on dry weight). The desired amount of inoculum, containing 8.9 +/- 0.8 mg fungal dry weight per ml culture, was then added to each bag using a sterile pipette. The bags were sealed using an electronic impulse sealer and well shaken. Fungal dry weight was determined by adding 30 ml of remaining homogenized inoculum to each of three dried and weighed 50 ml centrifuge tubes. The tubes were spun for 20 min at 6°C and 35,300g in a centrifuge (Beckman J2-HS, Fullerton, CA), weighed to the nearest 0.001 g, and the supernatant was decanted. Following oven-drying at 80°C for 12 hours, the tubes were re-weighed and fungal dry weights calculated.

Some of the straw was chopped and/or autoclaved prior to inoculation (Table 15). Prior to autoclaving, straw (100 g) was placed in aluminum pans and covered with aluminum foil. The pans were then autoclaved for 40 min at 121°C and transferred to a laminar flow cabinet overnight to dry. The straw was transferred from the pans into spawn bags and autoclaved for 20 min at 121°C. The bags were again placed in the laminar flow cabinet and immediately inoculated.

Table 15. Treatments (each performed in triplicate) used for inoculation of wheat straw in mushroom spawn bags.^a

Treatment #	Sterile, Distilled Water (ml per bag)	Inoculum (ml per bag)	Straw Autoclaved	Straw Chopped (0.7 in. screen)
1	60	30	No	No
2	60	30	No	Yes
3	60	30	Yes	No
4	60	60	No	No
5	60	120	No	No
6	60	120	Yes	Yes
7	0	120	No	No
8 ^b	20	60	No	No
9 ^b	50	30	No	No
10 ^b	65	15	No	No
11	60	NA ^c	No	No
12	NA ^d	NA ^d	No	No
13	0	0	No	No
14	0	0	Yes	No

a. Treatments #12, #13 and #14 were not incubated in mushroom spawn bags.

b. Sterile, distilled water and inoculum were mixed prior to application on straw; otherwise, water and inoculum were added consecutively.

c. 120 ml growth media were added instead of inoculum.

d. Treatment #12 consisted of 6% sodium hypochlorite (bleach). For a description of application, please see text.



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A control, consisting of 60 ml sterile, distilled water and 120 ml growth media, was included in the experiments (treatment #11, Table 15). A bleach (6% sodium hypochlorite) treatment (#12) was also included to determine its effectiveness in straw delignification. For the bleach treatment, 100 g of straw was added to each of four aluminum pans. One gallon of distilled water and 150 ml bleach were added to each pan. The straw in the pans was submerged in this solution for five hours, rinsed with distilled water twice and oven-dried.

All bags were incubated at 24°C and 65% relative humidity for three months. Following weight loss and moisture content (MC) measurements, the straw was ground in a laboratory mill (Thomas-Wileyâ, model 4, Thomas Scientific, Swedesboro, NJ) equipped with a 1 mm screen and stored at 4°C until used.

It was determined by visual observation that *P. ostreatus* successfully colonized straw only when sterilized straw was used. After approximately two weeks of incubation, abundant fluffy, white *P. ostreatus* mycelium was observed in the spawn bags containing sterilized straw (Figure 21A). It was demonstrated in additional experiments that sterilization of straw was successful only when the straw had been chopped prior to inoculation (data not shown).

In all treatments in which unsterilized straw was used, the straw was instead colonized by a plethora of microfungi (Figure 21B). No attempt was made to isolate and identify the fungi present since this was beyond the scope of this project.



A.



B.

Figure 21. Bags containing wheat straw, following three months of incubation with *P. ostreatus*. A: straw sterilized prior to inoculation; B: straw not sterilized prior to inoculation.

Wheat straw incubated in mushroom spawn bags was analyzed using weight loss and moisture content measurements, various types of microscopy, thermogravimetric analysis, and selected chemical assays. Light microscopy was used to investigate the distribution of *P. ostreatus* hyphae in sterilized straw. Transmission electron microscopy was employed to determine intact cell wall structure in rehydrated control wheat stems and modified cell wall structure of sterilized, *P. ostreatus* treated wheat stems.

Thermogravimetric analysis (TGA) was used to determine if a change in the chemical composition due to fungal degradation of the straw had an effect on its thermal stability. Thermogravimetric analysis is a technique for measuring the weight loss of a substance as a function of temperature.



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It has been used to evaluate fungal degradation of wood (Beall et al. 1976) and to identify changes in the components of mushroom and straw compost (Sharma 1990 and 1996, Blanco and Almendros 1994 and 1997).

The chemical analysis targeted lignin, cellulose and hemicelluloses. We expected that fungal treatment of sterilized straw with *P. ostreatus* would result in reduction of lignin and hemicelluloses in the substrate without much cellulose removal.

Wheat Straw Weight Loss and Moisture Content

At the end of incubation, the straw was weighed, oven-dried until constant weight was reached and re-weighed to determine dry weight and moisture content (MC). Moisture content was calculated using the following equation:

$$MC = \frac{W_w - W_d}{W_d} \cdot 100 (\%)$$

where W_w = wet weight of straw (g) and W_d = dry weight of straw (g).

The effectiveness of fungal degradation was estimated as the weight loss (on a dry-weight basis) over the three-month incubation period.

Weight loss was evident in all treatments, except in # 12 because this treatment (bleach) did not involve any inoculation and incubation. It should be stressed that a certain amount of water evaporation contributed to weight loss since the spawn bags did not completely inhibit moisture loss of the straw during incubation. The feasibility of using weight loss as an indicator of straw degradation was further compromised by the accumulation of *P. ostreatus*-mycelia in bags of treatment #6. Fungal mycelia on sterilized straw could not be separated from the substrate following incubation. In conclusion, weight loss is only of limited value to measure the effectiveness of straw degradation.

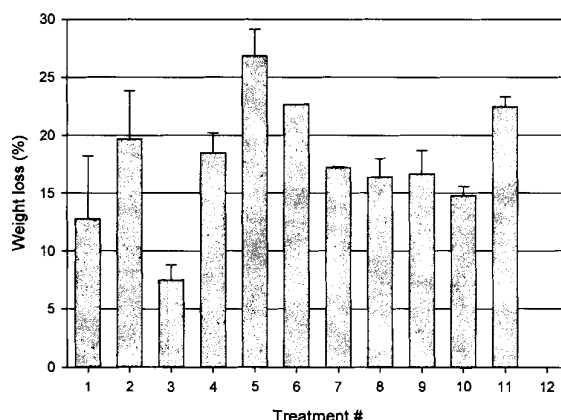


Figure 22. Weight losses of wheat straw obtained with different treatments (see Table 15 for description of treatments). Note that weight loss with treatment #12 was not determined and that weight loss of #6 was determined with one replicate only (no standard deviation shown).

The highest straw weight loss (27%) was obtained with treatment #5 which involved unsterilized straw (Figure 22). However, weight loss of straw treated with growth media (#11) was as high or higher than weight loss of unsterilized, treated straw (Figure 22), indicating that inoculation of unsterilized straw with *P. ostreatus* was not effective.

It appears that high weight losses achieved with treatments #5, #6 and #11 were due to comparatively high straw MC (more than 150%) after inoculation (Figure 23). Therefore, a straw MC of 150% after inoculation allows for optimum fungal degradation of straw in the mushroom spawn bags.



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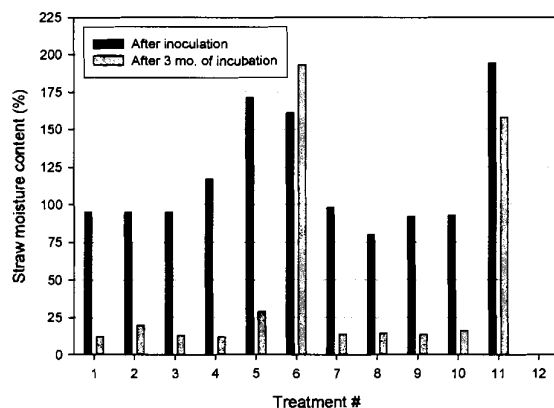


Figure 23. Moisture content (MC) of straw obtained with different treatments (see Table 15 for description of treatments), following inoculation and three months of incubation. Note that MC following incubation was not determined for treatment #11. MC of straw with treatment #12 was also not determined.

Wheat Straw Light and Transmission Electron Microscopy Analysis

Light and transmission electron microscopy were performed on sterilized, inoculated straw specimens from the mushroom spawn bags and untreated control samples. Inoculated wheat straw and control specimens were sliced into small (1 – 2 mm long segments) and immersed overnight in a chilled (4°C) fixative solution containing 0.5% (v/v) glutaraldehyde (Ted Pella Inc., Redding, CA), 2% (v/v) paraformaldehyde (Ted Pella Inc., Redding, CA), and 50 mM PIPES buffer (Research Organics, Cleveland, OH), pH 7.3. Specimens were then dehydrated in a graded ethanol series, infiltrated with LR White resin (Ted Pella Inc., Redding, CA), and allowed to polymerize overnight at 50°C. Semi-thin sections (0.5 – 1 µm) for light microscopy were cut with glass knives, and stained with 1% (w/v) Safranin O (Ted Pella Inc., Redding, CA) or

1% (w/v) toluidine blue (Ted Pella Inc., Redding, CA), and viewed at 1000x on an Olympus BH compound light microscope (Olympus Optical Co., Ltd., Tokyo, Japan). Digital images were collected with a Pulnix CCD camera (JAI Pulnix Inc., Sunnyvale, CA). Thin sectioning for transmission electron microscopy was accomplished using a diamond knife and an ultramicrotome (Ultracut R, Leica Microsystems, Vienna, Austria). Silver sections were collected on uncoated 300 mesh nickel grids. Sections were stained for 12 min with a uranyl acetate (UA)-KMnO₄ solution consisting of three parts 2% aqueous UA (Ted Pella Inc., Redding, CA), and one part 1% KMnO₄ (Mallinckrodt Inc., St. Louis, MO), mixed and filtered immediately prior to staining. Stained sections were observed with an electron microscope (Jeol JEM 1200EX, JEOL, Tokyo, Japan) at the Washington State University Electron Microscopy Center, and photographed with Kodak electron microscopy film.

Light and electron microscopy revealed that the effects of *P. ostreatus* growth in sterilized, inoculated straw samples were spread throughout the wheat stem tissues. All tissues from each specimen examined showed effects of fungal treatment. Light microscopy revealed that the wheat stems of the control sample showed little evidence of cellular distortion caused by drying and re-hydration of the straw, indicating that the cell walls of the sclerified storage parenchyma retained sufficient rigidity to resist collapse during dehydration (Figure 24A). In contrast, the equivalent cells of the *P. ostreatus* treated samples appeared less rounded, and often distorted (Figure 24C). Also, the nacreous cell walls of the conducting phloem cells were completely removed from all vascular bundles in all fungal treatment samples examined, indicating that the effects of fungal treatment were widespread throughout the samples (compare Figures 24B and 24D). Figure 24E shows hyphae within sclerified parenchyma cells of *Pleurotus ostreatus* treated wheat straw in higher magnification.



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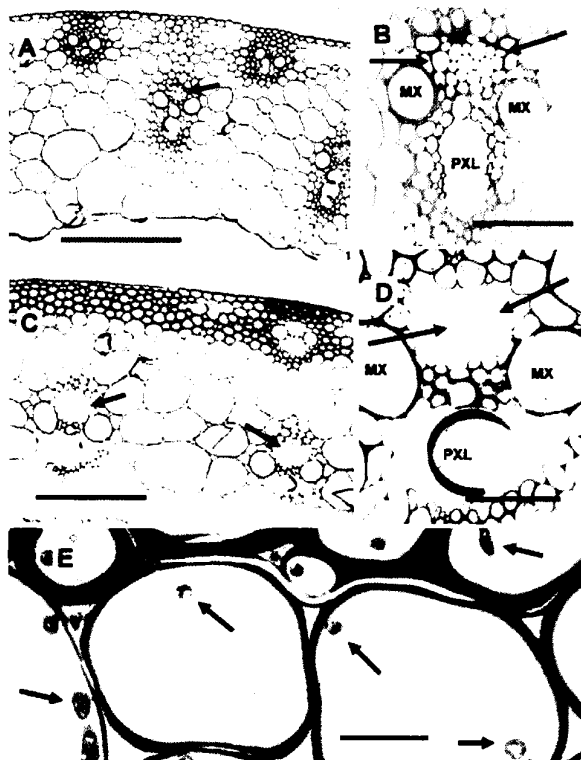


Figure 24. Light microscope images comparing *P. ostreatus* treated and untreated straw samples for the distribution of *P. ostreatus* hyphae and tissue-level effects of fungal treatment. (A) Rehydrated control wheat straw cross section showing rounded, apparently structurally well preserved sclerified parenchyma cells. Arrow indicates the region of conducting phloem cells (sieve tube members and companion cells) within a vascular bundle. Bar = 200 µm. (B) A higher magnification of a vascular bundle from a control sample. Nacreous cell walls of the conducting phloem remain intact (arrows). MX = metaxylem vessel element. PXL = protoxylem lacuna. Bar = 50 µm. (C) Cross section of a wheat stem from a *P. ostreatus* treatment sample showing less rounded cells than the control samples, possibly indicating widespread weakening of cell walls. Hyphal filaments can be seen in nearly every cell of all tissues present. Arrows indicate spaces in vascular bundles where the residual cell walls of conducting phloem cells have been removed by fungal treatment. Bar = 200 µm. (D) A higher magnification of a vascular bundle from a fungal treatment sample. Arrows indicate a space where fungal enzymes have removed the nacreous walls of conducting phloem cells. MX = metaxylem vessel. PXL = protoxylem lacuna. Bar = 50 µm. (E) A higher magnification showing hyphae (arrows) within sclerified parenchyma cells of *Pleurotus ostreatus* treated wheat straw. Bar = 10 µm.

Electron microscopy revealed evidence for cell wall modification by fungal enzymes. Control samples (Figure 25A-D) showed that cells walls of sclerenchyma fibers (Figure 25A,C) and sclerified parenchyma (Figure 25B,D) contained numerous darkly staining fine striations representing differential deposition of cell wall polymers, possibly lignin. These cell walls were smooth along the surface bordering the cell lumen, and the cells contained little residual cellular debris. In contrast, cell walls of *P. ostreatus* treated samples (Figure 26) appeared more uniformly stained, with very few fine striations, indicating a possible loss of cell wall polymers with exposure to fungal enzymes. Numerous fungal hyphae were seen within the lumen of all cell types examined. The cells also contained a granular slime sheath that coated both the fungal hyphae and the cell walls of the re-hydrated wheat stems (Figure 26A-D). A hyphal sheath, consisting of a thin glucan layer, was previously observed for a closely related fungal species, *P. eryngii* (Barrasa et al. 1998). Hyphal sheaths have been observed around many wood decay fungi (Palmer et al. 1983a and b, Highley et al. 1983, Ruel and Joseleau 1991, Green et al. 1992, Jellison et al. 1997) and sapstaining fungi (Schmid and Liese 1965, Gharibian et al. 1996, Ouellette et al. 1999, Schirp 2001). The hyphal sheath may protect



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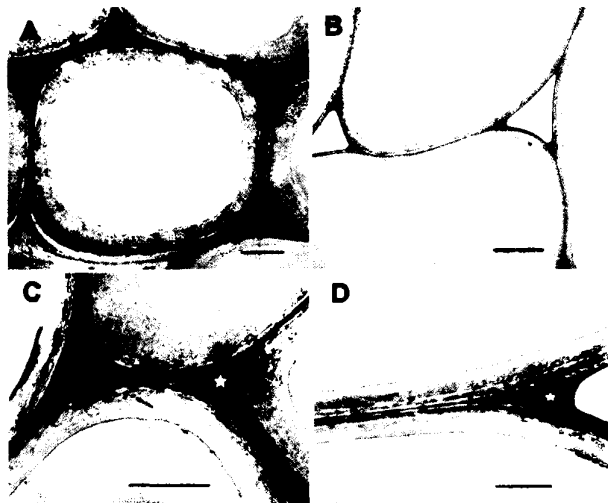


Figure 25. Transmission electron micrographs showing the intact cell wall structure and cell wall striations present in rehydrated control wheat stems. (A) Low magnification cross section of a sclerenchyma fiber showing a smooth cell wall surface at the cell lumen, numerous fine striations in the secondary wall and a darkly staining middle lamella. Bar = 2 μ m. (B) Low magnification cross section showing the cell walls of several sclerified storage parenchyma cells. Cell walls are clearly defined with a smooth interior surface. Bar = 5 μ m. (C) A higher magnification of sclerenchyma fiber cell walls. Note the darkly staining middle lamella (star) and the fine cell wall striations (arrow). Bar = 1 μ m. (D) Higher magnification of sclerified parenchyma cell walls. Note the smooth cell wall boundaries, the fine striations within the secondary walls, and the darkly staining middle lamella. Bar = 1 μ m.

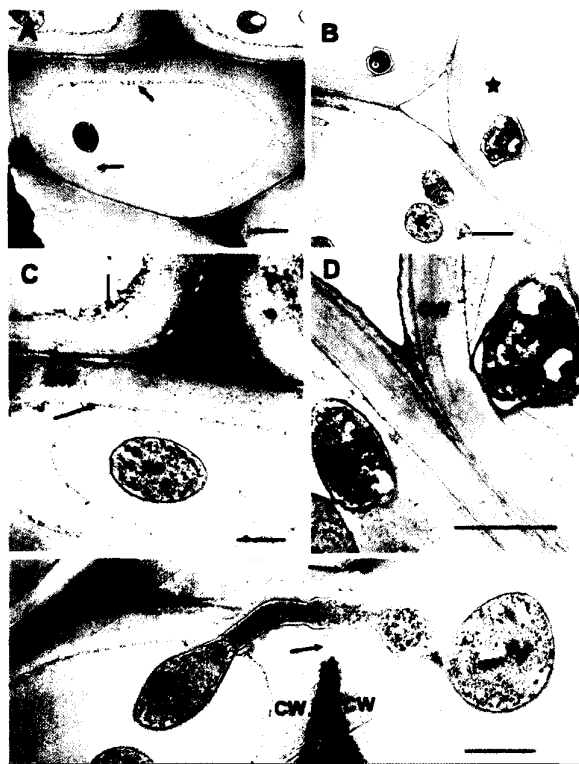


Figure 26. Transmission electron micrographs showing the modified cell wall structure of *Pleurotus ostreatus* treated wheat stems. (A) Low magnification of sclerenchyma fibers containing *Pleurotus* hyphae (P) showing a cell wall with relatively few cell wall striations and with a granular appearing material coating the interior surface (arrows). Bar = 2 μ m. (B) A low magnification showing cells walls of sclerified parenchyma cells. Each cell contains numerous *Pleurotus* hyphal filaments (P) surrounded by a coating material (star) apparently secreted by the fungi. Bar = 2 μ m. (C) Higher magnification of sclerenchyma fibers containing *Pleurotus* hyphae (P). A granular material coats the interior surface of the cells (arrows) and the cell walls contain relatively few fine striations. Bar = 1 μ m. (D) A higher magnification of the sclerified parenchyma shown in (B). Note the secreted material surrounding the hyphae (P), the cell walls (CW) with few striations, and relatively light staining of the middle lamella. Bar = 2 μ m. (E) A hyphal filament traversing thick cell walls (CW) of sclerenchyma fibers. Note the lack of staining of the middle lamella near the region of cell wall penetration (arrows) indicating removal of pectin by fungal enzymes. Bar = 2 μ m.



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extracellular enzymes from inactivation and retain them close to the fungal hyphae. Optimal pH levels of 7 to 9 have been reported for xylanases and pectinases, therefore, the extracellular sheath may also serve to maintain an environment favorable to enzyme activity since wood is acidic, with a typical pH of 4–5 (Gharibian et al. 1996).

Hyphae of *P. ostreatus* were often seen traversing cell walls (Figure 26E). The pathways between cells may have begun as natural pits, or as cracks caused by cell wall drying, but the cell wall at these sites show evident cell wall modification from fungal enzyme activity.

Wheat Straw Thermogravimetric Analysis (TGA)

Ground straw samples were dried in a vacuum oven at room temperature overnight to obtain a straw MC between 5 and 7%. Thermogravimetric analysis was performed using a simultaneous thermal analyzer (Rheometric Scientific STA 625, Piscataway, NJ). Straw samples of approximately 4 mg weight were heated in an aluminum pan (Rheometric Scientific L7168 2 mm, Piscataway, NJ) to 580°C at a heating rate of 60°C per minute. The maximum temperature (580°C) was held for 20 min, followed by cooling to 30°C at a rate of 60°C per minute. Each sample was run in duplicate. After each TGA-run, the data obtained were converted from the Rheometric software (RSI Orchestrator, Version V6.5.5) into an Excel-file, and weight losses were calculated based on the original sample weights with correction for the buoyancy effect of the air.

Initial substrate weight losses in the low temperature range of up to 100°C can be attributed to dehydration. Thermogravimetric analysis showed that the highest weight losses of straw occurred in the temperature range between 200°C and 350°C, followed by a less active pyrolysis stage, which ranged from 350°C to 600°C (Figures 27A-B). This result is comparable to findings by Sharma (1990) who investigated straw degraded by *P. ostreatus* after 20 and 40 days of incubation.

In the temperature ranges between 260°C and 330°C as well as between 380°C and 580°C, the relative weight losses of sterilized straw inoculated with *P. ostreatus* were higher than those of unsterilized, inoculated straw (Figure 27A). This indicates that straw, which has been sterilized prior to inoculation, is thermally less stable than unsterilized, treated straw. Therefore, a higher level of fungal degradation was achieved with sterilized straw. However, it has to be taken into consideration that a large amount of fungal biomass was present in the sterilized and treated straw. This may have had an influence on the thermal behavior of the tested samples.

It is apparent that the sterilization procedure per se reduced the thermal stability of the straw (Figure 27B). Thermogravimetric analysis also demonstrated that there is no difference in the thermal stability of treated straw whether the straw had been chopped to a length of 1.8 cm prior to fungal treatment or not (Figure 27C).

In summary, TGA indicated that sterilized, treated straw possesses reduced thermal stability in comparison to unsterilized, treated straw. This demonstrates, in turn, that fungal degradation of the straw was more pronounced in sterilized straw than in unsterilized straw. Fungal degradation of a lignocellulosic substrate like wood or straw results in enzymatic depolymerization of this substance, suggesting that the degraded material has reduced thermal stability (Beall et al. 1976).



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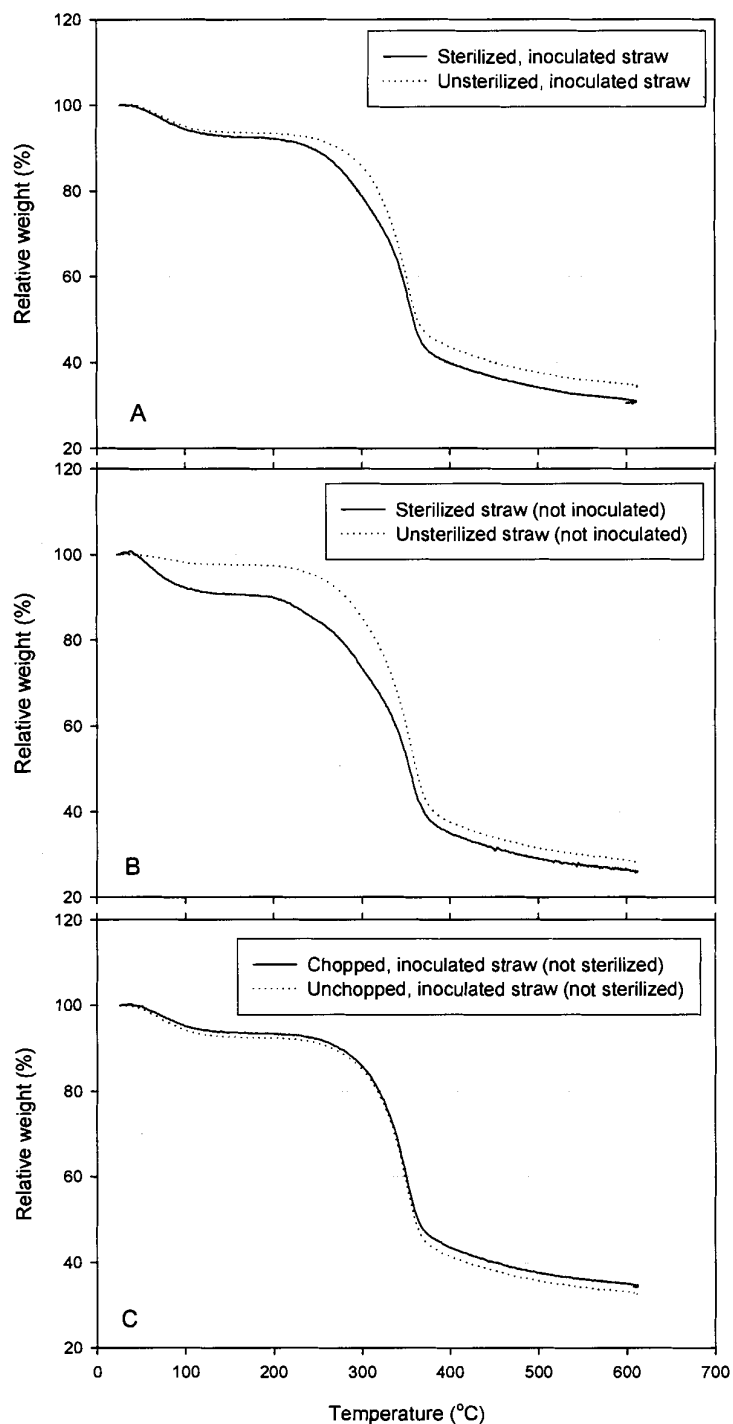


Figure 27. Thermal stability of straw treatments. A: effect of sterilization prior to inoculation with *P. ostreatus*; B: effect of sterilization; C: effect of straw stem length.



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Wheat Straw Chemical Analysis

Carbohydrate and lignin analyses were performed on approximately 350 mg of oven-dried material of treatments #4, #5, #6, #11, #12, #13 and #14 from the spawn-bags (Table 15). Individual monosaccharides (araban, xylan, mannan, galactan and glucan) were separated on a glass column packed with 3% cyanopropyl silicone (SP-2340, (Supelco, Bellefonte, PA) in a gas chromatograph (Hewlett-Packard 5890, Series II, Wilmington, DE) at 205°C following sulfuric acid hydrolysis according to TAPPI Method T 249 cm-85 (TAPPI 1985). Acid-insoluble Klason lignin was determined according to Effland (1977). Individual samples were analyzed in duplicate.

Monosaccharide and acid-insoluble Klason lignin content of treated straw was determined. The results of carbohydrate analysis are presented in Table 16. Glucan is the dominant carbohydrate which mostly makes up the cellulose. However, a portion of the glucan is combined with mannan in the hemicellulose fraction. The ratio between glucan and mannan is 1:2, therefore, hemicellulose content for each sample was estimated as the sum of all non-glucose sugars plus 1/2 of the mannan fraction (Table 17). Cellulose content was estimated as the sum of the glucan value minus 1/2 of the mannan fraction. The dominant non-glucose carbohydrate in hardwoods as well as in wheat straw is xylan.

Table 16. Carbohydrate composition of straw treatments, based on % of oven-dried straw.

Treatment	% ^a				
	Arabinan	Xylan	Mannan	Galactan	Glucan
Sterilized, inoculated (#6)	2.4	17.6	2.1	0.7	33.7
Sterilized, not inoculated	2.7	14.4	1.3	0.8	27.4
Not sterilized, inoculated (#5) ^b	2.3	16.2	1.3	0.9	28.3
Not sterilized, inoculated (#4) ^c	2.5	18.4	1.3	0.8	31.1
Not sterilized, not treated	3.0	18.8	1.2	0.8	32.2
Growth media (#11)	2.6	18.0	1.2	1.1	31.6
Bleach (#12)	3.3	17.5	1.1	0.9	31.7

a. Each value represents the average of two chromatographic runs of one sample. Standard deviation for all values was between 0 and 0.14.

b. 120 ml inoculum.

c. 60 ml inoculum.

Table 17. Cellulose, hemicellulose and lignin composition of straw treatments, based on % of oven-dried straw.

Treatment	%		
	Hemicellulose ^a	Cellulose ^a	Lignin ^b
Sterilized, inoculated (#6)	23.8	32.7	17.4
Sterilized, not inoculated	19.9	26.8	22.8
Not sterilized, inoculated (#5) ^c	21.2	27.7	28.4
Not sterilized, inoculated (#4) ^d	23.6	30.5	25.9
Not sterilized, not inoculated	24.3	31.6	21.3
Growth media (#11)	23.5	31.0	25.4
Bleach (#12)	23.4	31.1	22.6

a. Each value represents the average of two chromatographic runs of one sample. Standard deviation for all values was between 0 and 0.18.

b. Each value represents the result for one sample.

c. 120 ml inoculum.

d. 60 ml inoculum.



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Unsterilized, untreated wheat straw contained 21.3% lignin and therefore less lignin than most wood species (Fengel and Wegener 1989). The amount of lignin in straw determined in this study is comparable to results by Muller (1960) who found 18.2% lignin in the internodes of summer wheat and 20.2% lignin in winter wheat (values for lignin in nodes were similar for summer wheat and slightly lower for winter wheat). Harper and Lynch (1981) also determined lignin content in different anatomical parts of wheat straw and found 14.2% lignin in internodes and 16.7% in node cores.

The greatest reduction in lignin was achieved in the sterilized, inoculated straw (Treatment #6, Table 17). Sterilized, inoculated straw contained 17.4% lignin and therefore 24% less lignin than sterilized, non-inoculated straw (22.8%). Inoculation of unsterilized straw with *P. ostreatus* did not reduce the amount of lignin in the straw, and neither did the addition of growth media or bleach (Table 17). In addition, fungal treatment of sterilized straw did not reduce the amount of cellulose and hemicelluloses in the straw and was therefore shown to be selective for lignin. Inoculation of unsterilized straw with *P. ostreatus* reduced cellulose by up to 12% and hemicelluloses by up to 13%. Fungal treatment of unsterilized straw with *P. ostreatus* was therefore not selective for lignin.

The total sum of the hemicellulose, cellulose and lignin fractions is less than 100% (Table 17) due to extractives, crude protein, ash and silica present in the straw. Overall, the lowest amount of hemicellulose was determined in sterilized, non-inoculated straw. This can be explained with the autoclaving procedure, which is comparable to steam-explosion treatment, albeit performed at lower pressure. It has long been known that steam-explosion solubilizes large amounts of sugars in wood (Saddler et al. 1982) and wheat straw (Vallander and Eriksson 1985). In fact, some monosaccharides were likely lost in all straw samples during the autoclaving step, which is part of the sulfuric acid hydrolysis.

Task 3b – Performance Evaluation of Straw-Plastic Composites

Investigators: Anke Schirp,¹ Frank J. Loge,¹ Michael P. Wolcott,¹ Karl Englund,¹ David N. Thompson,² Steven Aust,³ Paul Swaner,³ David Dostal,⁴ and Matthew Nelson⁵

Performing Institutions: ¹Washington State University, ²Idaho National Engineering and Environmental Laboratory, ³Utah State University, ⁴Strandex Corporation, and ⁵Integrated Paper Services

Acknowledgement: We would like to thank Prof. Steven Aust and Paul Swaner of Utah State University for maintaining and providing the fungal cultures of *P. ostreatus*; David Dostal for extrusion of the wood-plastic formulations; Strandex®, Madison, WI, for their cooperation in the large-scale pilot trial; Glenn Turner for providing micrographs; Matthew Nelson of Integrated Paper Services, Appleton, WI, for performing chemical analysis of straw; and Ryan Roberts and Matthew Chastagner for help with some practical aspects of the research in this study.

The types of analyses used to evaluate the material properties of the straw-plastic composites varied according to the source of degraded straw (mushroom spawn bags, small-scale trial, or large-scale trial). Composite materials manufactured with straw incubated in mushroom spawn bags were analyzed with dynamic mechanical analysis (DMA). Composite materials manufactured with straw obtained from the small-scale trial were analyzed for flexural strength and selected physical properties.



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Straw-Plastic Composites based on Straw from Mushroom Spawn Bags

As described above, straw produced with the mushroom spawn bag system at Washington State University was used for these tests. Treatments #5, #6, #13, #14 and a control consisting of 60-mesh Southern yellow pine flour were compounded and processed into thermoplastic composites. Sixty percent (by weight) of individual fiber treatments and 40% (by weight) of high-density polyethylene powder (Equistar Chemical LB010000, Houston, TX) were thoroughly mixed by shaking in a plastic bag and then compounded into pellets at 180°C and 20 rpm in a Haake PolyLab System (Rheocord 300p and Rheomix 600p, Thermo Haake, Karlsruhe, Germany), equipped with roller rotors. Processing time was approximately 5 min for each batch. Pellets were added to a screw-driven capillary extrusion rheometer (Acer 2000, Rheometric Scientific, Piscataway, NJ), equipped with a home-made die, soaked for 30 min at 180°C, and injection-molded into samples (nominal dimensions: 1.4 mm thickness, 6 mm width, 45 mm length) for dynamic mechanical analysis (DMA). Density of DMA-specimens was 1.34 +/- 0.02 g/cm³ for all samples with the exception of the sterilized and treated samples (treatment #6; 1.39 +/- 0.01 g/cm³) and the sterilized and untreated controls (0.93 +/- 0.04 g/cm³).

Straw-plastic composites made from straw incubated in mushroom spawn bags were analyzed using dynamic mechanical analysis. DMA was conducted in dual cantilever mode in a Rheometrics RSA II solids analyzer (Piscataway, NJ). Initially, dynamic strain sweep tests from 10⁻⁴ to 10⁻³ were run at -50, 25 and 100°C to ensure linearity throughout the test. Dynamic temperature scans from -50°C to +100°C were conducted at sequential frequencies of 0.1 Hz, 1 Hz and 10 Hz and a strain of 10⁻⁴. In all experiments, the heating rate was 2°C per minute, and the soak time was one minute.

The activation energy for the α -transition of the straw-plastic composite (SPC) was calculated using the Arrhenius equation (Turi 1997):

$$k = A_e \exp\left[-\frac{E_a}{RT}\right]$$

where k = rate constant or test frequency; A_e = frequency factor; R = ideal gas constant, 8.314 J/(mol K); T = temperature (K); E_a = activation energy. The peak temperatures of E" at different frequencies were calculated using software (RSI Orchestrator, version V6.5.5, Rheometric Scientific, Piscataway, NJ).

Statistical analysis was conducted to determine if the differences in activation energy required for α -transition were significant at a 95% confidence level for (1) sterilized, untreated and sterilized, treated straw; and (2) unsterilized, untreated and unsterilized, treated straw. In each case, the differences between the sample means were compared to the variation due to random error.

Dynamic mechanical analysis has been used extensively to characterize synthetic composite materials as well as wood and wood products (e.g., Salmén 1984, Atack 1981, Kelley et al. 1987, Birkinshaw et al. 1989, Rials and Wolcott 1998, Hristov and Vasileva 2003, Son et al. 2003). Dynamic mechanical analysis can potentially provide valuable molecular and morphological information about a material in the solid state by subjecting it to dynamic loads over a broad range of temperature and frequency (Saini and Shenoy 1985). During measurement, a sinusoidal strain is applied to the sample, while measuring the sinusoidal stress response. A portion of the response output is in phase with the strain input and



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represents the energy stored in the material or the elastic component (E' , storage modulus). The remaining response is out of phase with the strain and represents the energy dissipated by the material or the viscous component (E'' , loss modulus).

Figure 28 shows the temperature dependence of the storage and loss moduli of representative SPC specimens incorporating straw with various treatments at a frequency of 1 Hz. With increasing temperature, a drop in storage modulus occurred. We also determined that with increasing frequency, higher values for the storage modulus were obtained (data not shown).

The highest values for E' were obtained when SPCs were manufactured with sterilized and treated straw (Figure 28A). This indicates an improvement in the straw-polyethylene interphase of a SPC incorporating sterilized straw compared to a SPC based on unsterilized straw.

A phase transition is noted by a peak in the loss modulus between 30 and 65°C, depending on the frequency and straw treatment (Figure 28B). It is desirable to assign this transition to a particular phenomenon to interpret the role of straw treatment on the composite performance. Relaxation transitions in polymers are labeled as α , β , γ etc. in alphabetical order with decreasing temperature (Ward and Hadley 1993). The transitions in high- and low-density polyethylene (PE) have been investigated extensively (Kosfeld et al. 1981, Saini and Shenoy 1985, Ward and Hadley 1993). PE shows clearly resolved peaks for α -, β - and γ -transitions (Ward and Hadley 1993). The γ -transition corresponds to the glass transition temperature of PE (Kosfeld et al. 1981, Saini and Shenoy 1985) whereas the α -transition corresponds to the molecular segmental motion in the crystalline phase, i.e. chain rotation (Saini and Shenoy 1985). The α -relaxation in PE is often considerably modified, appearing to consist of at least two processes with different activation energies (Ward and Hadley 1993). The number of publications in which transitions in fiber-filled high-density polyethylene (HDPE) were identified is limited (Wang et al. 2003, Simonsen and Rials 1996, Balasuriya et al. 2003). Wang et al. (2003) and Balasuriya et al. (2003) identified the HDPE γ -transition at -115°C in wood-filled HDPE. Simonsen and Rials (1996) investigated blends of recycled plastics (HDPE and polystyrene) and recycled wood fiber and identified the α -transition of polyethylene at 55°C. Balasuriya et al. (2003) report that the α -transition of HDPE shifts from 42.9°C to a higher temperature with increasing (recycled) wood content.

Activation energies for the α -transition of HDPE were calculated using Arrhenius plots (Figure 29). According to the Arrhenius equation, a plot of the natural logarithm of the frequency versus $1/T$ (K^{-1}) provides a straight line with a slope proportional to the activation energy. For composites, high activation energies are associated with large degrees of interactions between polymer matrix and filler. In general, activation energies obtained for the α -transition in our SPC are comparable to literature values for various polyethylenes and polyethylene-based composites (Matsuo et al. 2003, Vaisman et al. 2003, Pegoretti et al. 2000).

Activation energy of an SPC based on sterilized, untreated straw was 125 kJ/mol or 9% higher than sterilized, treated straw (113 kJ/mol). This difference was determined to be statistically significant at a 95% confidence level and confirmed that straw sterilization prior to fungal inoculation improved interfacial adhesion and stiffness of the material. In contrast, there was no significant difference at a 95% confidence level between treated (nonselectively degraded) and untreated samples when the straw was not sterilized prior to inoculation. Unsterilized straw that was selectively degraded in the laboratory at INEEL was not tested.

At present, we can only speculate which mechanism may be responsible for the interfacial improvement in SPC based on sterilized straw. It is possible that fungal inoculation of straw prior to mixing with a thermoplastic matrix and compounding resulted in improved bonding between straw and plastic.



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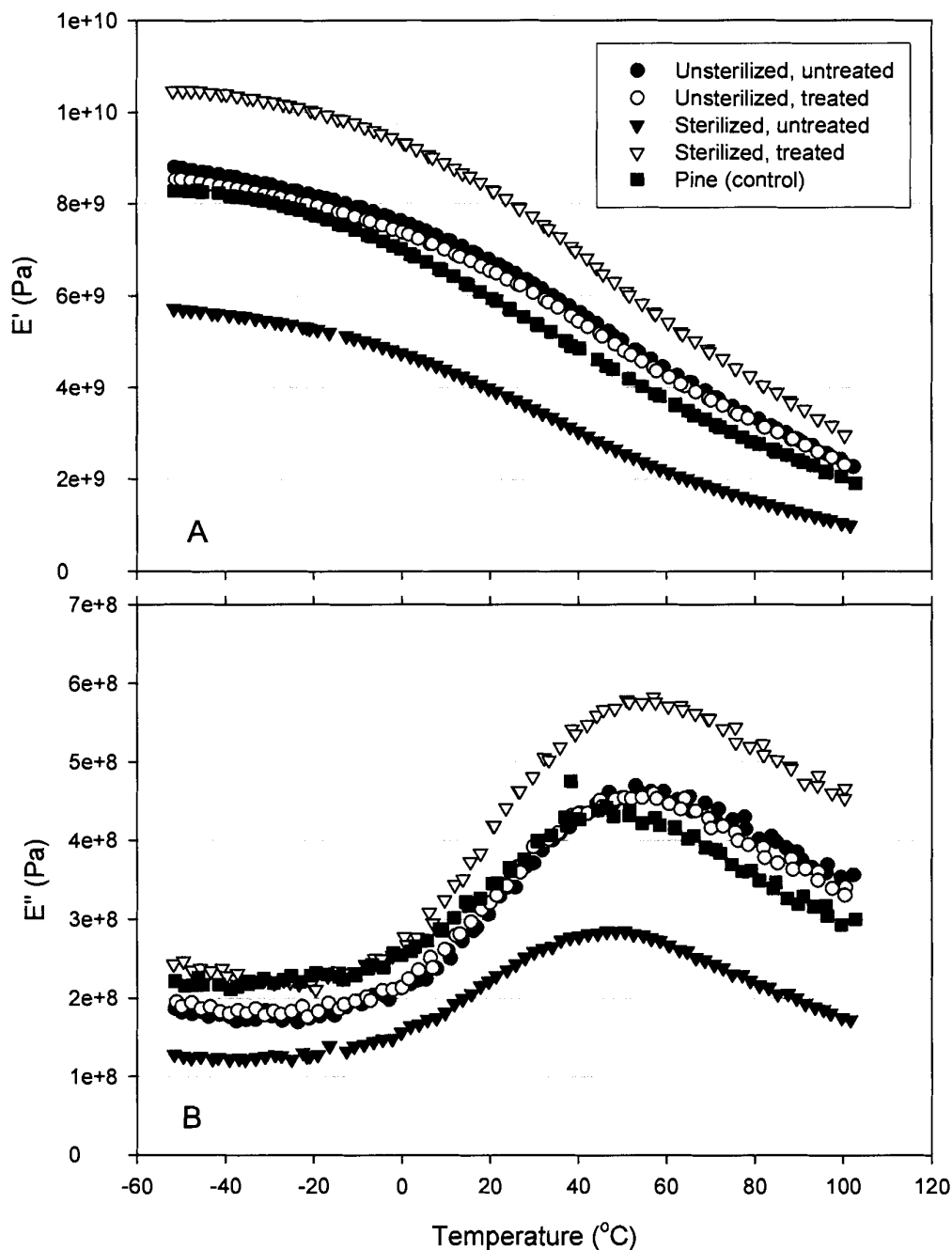


Figure 28. Dynamic mechanical analysis of injection-molded straw-plastic composites based on straw from mushroom spawn bags and one wood-plastic composite based on unmodified pine flour (frequency 1 Hz). (A) Storage modulus (E') as a function of temperature; (B) Loss modulus (E'') as a function of temperature.



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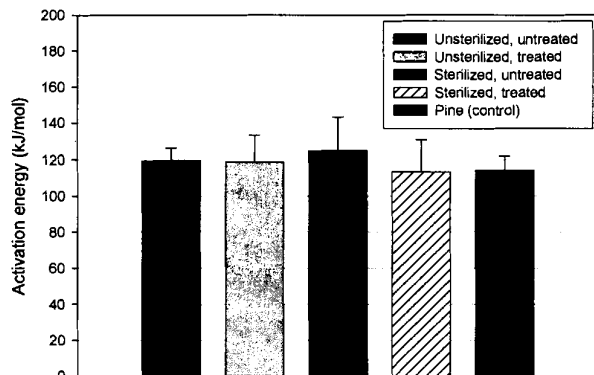


Figure 29. Activation energies for α -transition of HDPE in injection-molded straw-plastic composites based on straw from mushroom spawn bags and one wood-plastic composite based on unmodified pine flour. Data were collected in frequency-temperature scans performed from -50°C to $+100^{\circ}\text{C}$ at simultaneous frequencies of 0.1 Hz, 1 Hz and 10 Hz. Each bar represents the average of three values for activation energy obtained in individual frequency-temperature scans. Striped bars indicate that treated and untreated SPC were significantly different at a 95% confidence level when the straw was sterilized prior to fungal inoculation.

Straw-Plastic Composites based on Straw from Small-Scale Pilot Trial

The straw was inoculated at the Idaho National Environmental Engineering Laboratory (INEEL) by spraying liquid inoculum of known optical density (OD) and biomass onto fresh stems using a pressurized garden sprayer (Houghton et al. 2004). Straw MC was adjusted as desired, and the straw was packed into columns fabricated from glass process pipe. Following six weeks of incubation, the treated stems were removed from the glass columns and mixed thoroughly by hand at 1:10 (w/w) with fresh, air-dried, uninoculated straw stems. Straw MC was brought to 1.6 g of water per g of straw as fresh stems were mixed with the inoculated straw from the columns. This straw was then packed into 208.5 L-drums at about 7.5 kg dry weight of inoculated straw per drum and incubated for six and 12 weeks, respectively. Humidified air was supplied to each drum during incubation. Following incubation, samples were taken for chemical analysis, and the remaining material was shipped to the Wood Materials and Engineering Laboratory at

Washington State University for extrusion and subsequent evaluation of material properties. Straw samples were referred to as neat (untreated), Degrade 1 (treated for six weeks), and Degrade 2 (treated for 12 weeks).

Visual inspection of the incubated straw used in both pilot trials showed that *P. ostreatus* was not the dominating microorganism. Therefore, any straw degradation observed in the pilot trials was attributed to the degradative activity of a multitude of fungi comprising those which were naturally present on the straw prior to treatment, the inoculated fungus, *P. ostreatus*, as well as any microorganisms introduced during incubation. Nonselective degradation of the straw was observed (Houghton et al. 2004), confirming that *P. ostreatus* did not dominate the culture.

It was shown in the laboratory tests that, with homogeneous inoculation of actively growing nitrogen limited mycelia, it was possible to achieve successful growth of *P. ostreatus* on unsterile straw. This result has also been shown on unsterile wheat straw using a different *Pleurotus* species (Suzuki et al., 1995). However, we did not achieve this result using solids mixing techniques at a larger scale, and so further research will be needed to determine appropriate inoculation strategies and methods for larger-scale systems. In addition, a spore-forming fungus such as *Phanerochaete chrysosporium*, which has already been successfully applied in biopulping (Scott and Akhtar 2001) and bioremediation (Aust 1990), may be a more promising candidate for this purpose. With a spore-forming fungus, simultaneous germination of large amounts of spores and subsequent dominance of the inoculated fungus over existing microorganisms in a nonsterile environment may be achievable. A drawback of *P. chrysosporium* in our application, however, would be its nonselective degradation of lignocellulose (Breen and Singleton, 1999).



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In biopulping, wood chips are commonly steam-sterilized prior to fungal inoculation (Scott and Akhtar 2001). It is recognized that sterilization of straw prior to fungal treatment increases overall costs, however, if a successful inoculation method cannot be developed, it may be necessary to achieve dominance of *P. ostreatus* and the desired straw characteristics. In addition, the implementation of a sterilization process may allow for reduction of the total amount of fungal inoculum required for straw treatment. This would in turn increase cost-efficiency of the treatment process.

To test these small pilot-scale straw degradation treatments, a fractional factorial design was created to evaluate how the formulation components affect product performance (Table 18). This design included duplicate runs of identical formulations. In addition, runs #2, #12 and #17 were also performed with Southern yellow pine-flour (60 mesh) as filler instead of wheat straw (Table 19). Composite formulations were prepared by initially hammer-milling the straw through a 0.69 mm-screen and oven-drying to 1% MC. The dried straw particles were then blended with various amounts of HDPE (Equistar LB010000, Houston, TX), polyester-based wax (Honeywell OP-100, Morristown, NJ), and coupling agent (maleic-anhydride-grafted polyethylene, Honeywell 575A, Morristown, NJ). The formulations were extruded with a 35-mm counter-rotating conical twin screw extruder (Cincinnati Milacron CMT 35, Batavia, OH) which produced a 9.53 x 38.1 mm² solid cross section.

Straw-plastic composites made from each formulation in the fractional factorial design (Table 18) using straw from the small-scale pilot trial were evaluated for flexural strength (ASTM D790-97), water sorption and thickness swell (ASTM D 1037, modified), and particle size distribution, using a Ro-Tap® sieve shaker (W. S. Tyler, Mentor, OH), with screen sizes ranging from 0-830 µm.

Statistical significance of fungal treatment on modulus of rupture (MOR) and modulus of elasticity (MOE) in the small-scale pilot trial were determined using a General Linear Models Analysis of Variance (GLM ANOVA) approach with Number Cruncher Statistical Software (Kaysville, Utah). Statistical differences in MOE and MOR among three formulations using either pine or wheat as a filler (Table 20) were also analyzed using a GLM ANOVA approach with density as a covariate at an α -value of 0.05.

Fungal treatment did not have a statistically significant impact on either MOR or MOE at an α -value of 0.05. Density was found not to be a statistically significant covariant in the analyses.

In general, the most significant factor governing flexural strength (MOR) of the SPC was the amount of wheat straw incorporated in the formulation (Table 20). Flexural strength decreased with increasing amounts of wheat straw used when the formulation did not contain any coupling agent. Optimum flexural strength was obtained when 60–65% straw filler were used in the formulation. Flexural strength was improved with the incorporation of HDPE and MAPE in the formulations. The incorporation of a lubricant such as OP100 compromised MOR and MOE of the composite, as has been demonstrated by Harper (2003).

The most influential variable regarding flexural stiffness of the SPC was determined to be MAPE. The addition of a coupling agent in combination with low amounts of straw filler caused an increase in stiffness of the composite. Improvements in material strength with the addition of coupling agents may be the result of improved fiber-plastic interaction, better dispersion of the filler, or changes in the thermoplastic morphology (Harper and Wolcott 2004).



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Table 18. Formulations used in extrusion incorporating wheat straw from small-scale pilot trial^a and density, modulus of elasticity (MOE) and modulus of rupture (MOR) of straw-plastic composites. Values for components in formulation design represent weight-percentages required to make a 2-kg batch for extrusion.

Formulation Design (%)						Density (kg/m ³)		MOE (MPa)		MOR (Pa)	
Run#	Treatment	Wheat	HDPE ^b	MAPE ^c	Lubricant	Mean	% CV ^d	Mean	% CV ^d	Mean	% CV ^d
25	Degrade 1	55	38	4	3	1143	1.0	4117	4.3	31943	3.6
24	Degrade 1	55	41	4	0	1090	12.2	4159	8.0	37656	4.0
5	Degrade 1	55	42	0	3	1110	0.5	2678	8.8	23708	9.9
10	Degrade 1	55	42	0	3	1111	0.4	2411	21.9	21208	14.3
29	Degrade 1	55	45	0	0	1126	0.6	4072	5.6	32959	2.2
3	Degrade 1	60	36.25	3	0.75	1172	1.6	4713	7.4	35264	4.4
27	Degrade 1	60	36.25	3	0.75	1162	1.4	4562	6.3	34773	5.1
14	Degrade 1	60	38.25	1	0.75	1161	1.8	4610	6.0	34117	3.3
30	Degrade 1	60	38.25	1	0.75	1156	0.6	4261	6.4	31837	3.6
9	Degrade 1	70	26.75	1	2.25	1181	0.7	2782	5.2	19808	7.0
26	Degrade 1	75	18	4	3	1200	0.6	3205	7.5	17987	8.2
32	Degrade 1	75	18	4	3	1187	1.8	2694	15.4	16428	14.7
33	Degrade 1	75	21	4	0	1228	1.4	4500	13.5	28496	9.4
8	Degrade 1	75	22	0	3	1181	0.6	1524	27.9	11723	18.6
1	Degrade 2	55	38	4	3	1140	2.0	3616	14.3	30532	4.8
20	Degrade 2	55	38	4	3	1143	1.4	3621	7.8	31208	4.1
11	Degrade 2	55	41	4	0	1126	0.9	3507	5.7	34848	2.4
7	Degrade 2	55	42	0	3	1089	1.1	2425	9.7	22610	8.4
15	Degrade 2	55	45	0	0	1136	1.4	3608	8.1	32904	4.6
6	Degrade 2	65	35	0	0	1182	0.9	3772	11.9	27868	6.6
16	Degrade 2	65	35	0	0	1194	1.2	3671	10.8	26854	7.0
31	Degrade 2	75	18	4	3	1229	2.0	3067	6.1	18217	11.0
4	Degrade 2	75	21	4	0	1258	1.1	4343	13.8	26511	17.0
13	Degrade 2	75	22	0	3	1201	1.8	2263	10.5	14272	10.2
12	Neat	55	38	4	3	1127	0.4	4355	6.4	35025	3.8
17	Neat	55	41	4	0	1119	0.5	3260	14.7	35899	8.5
21	Neat	55	42	0	3	1063	0.7	2101	5.9	18991	8.7
18	Neat	55	45	0	0	1113	0.7	3733	6.7	29641	2.8
34	Neat	65	31	4	0	1183	1.0	5486	8.2	39602	4.4
22	Neat	65	31.5	2	1.5	1158	1.6	4717	10.0	30541	9.8
23	Neat	65	31.5	2	1.5	1156	0.6	4726	8.6	31827	3.8
19	Neat	65	32	0	3	1117	1.1	1562	16.4	15752	7.9
2	Neat	75	18	4	3	1192	0.5	3396	10.2	19943	8.3
28	Neat	75	25	0	0	1188	1.0	2650	14.1	17188	11.8

a. Five replicates were extruded per formulation.

b. High-density polyethylene.

c. Maleic-anhydride-grafted polyethylene.

d. Coefficient of variation.



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Table 19. Formulations used for large-scale extrusion runs with wheat straw and pine flour.

Component (weight %)	#1	#2
Wheat straw	65	58
High-density polyethylene (LB0100 00, Equistar)	35	31
Maleic-anhydride-grafted polyethylene (575A, Honeywell)	0	0
Lubricant (OP-100, Honeywell)	0	0
Commercial lubricant (zinc stearate, DLG-20B, Ferro)	0	2
Commercial lubricant (ethylene-bisstearamide, GE Specialty Chemicals)	0	1
Talc (Nicron 403, Luzenac)	0	6
Zinc borate (US Borax)	0	2

Table 20. Density, modulus of rupture (MOR) and modulus of elasticity (MOE) of three extruded formulations with untreated pine and wheat flour as fiber raw materials.

Run #	Formulation (weight %)				Properties (coefficient of variation)			
	Fiber	HDPE ^a	MAPE ^b	Lub ^c	Fiber Type	Density (kg/m ³)	MOE (MPa)	MOR (Pa)
2	75	18	4	3	Straw	1192 (0.52)	3396 (10.24)	19943 (8.31)
					Pine	1175 (0.94)	2995 (11.74)	19926 (10.45)
12	55	38	4	3	Straw	1127 (0.42)	4355 (6.35)	35025 (3.80)
					Pine	1136 (1.94)	3816 (11.45)	34864 (4.23)
17	55	41	4	0	Straw	1119 (0.52)	3260 (14.68)	35899 (8.45)
					Pine	1122 (0.69)	3235 (7.22)	35828 (3.57)

a. High-density polyethylene.

b. Maleic-anhydride-grafted polyethylene.

c. Lubricant.

With the exception of the formulation used in run # 12, MOE was not significantly different between straw- and pine-based composites at an α -value of 0.05 (Table 20). In addition, the modulus of rupture was not significantly different between straw- and pine-based composites at an α -value of 0.05, except for the formulation used in run #2 (Table 20).

From a practical point of view, straw- and wood-plastic composites appear very similar with regard to their mechanical performance. However, their water sorption and thickness swell strongly depend on the amount of filler used in the formulation (Figure 30a, b and 31a, b). In general, water sorption, thickness swell and dimensional instability of the composites increased with the incorporation of higher amounts of filler in the formulation. SPC specimens comprised of degraded straw demonstrated less resistance to the sorption of water than specimens in which neat straw or pine was used (Figure 30a, b). This may primarily reflect the fungal degradation of hydrophobic cell wall components (lignin and hemicelluloses) in treated straw, resulting in a relatively more hydrophilic substrate compared to neat straw. In addition, fungal treatment may have caused disruption of the waxy cuticle surrounding the straw, thus further reducing straw hydrophobicity and improving water sorption in the SPC.

The extruding performance of some of the runs with MAPE and high levels of HDPE (#12, #17, #18 and #34) was marginal at best. The extrudate for these runs exhibited a "snake skin" or rough surface appearance (Figure 32). This was not due to incorporation of wheat straw since similar results were found when wood flour was used in place of the wheat straw in runs #12 and #17.



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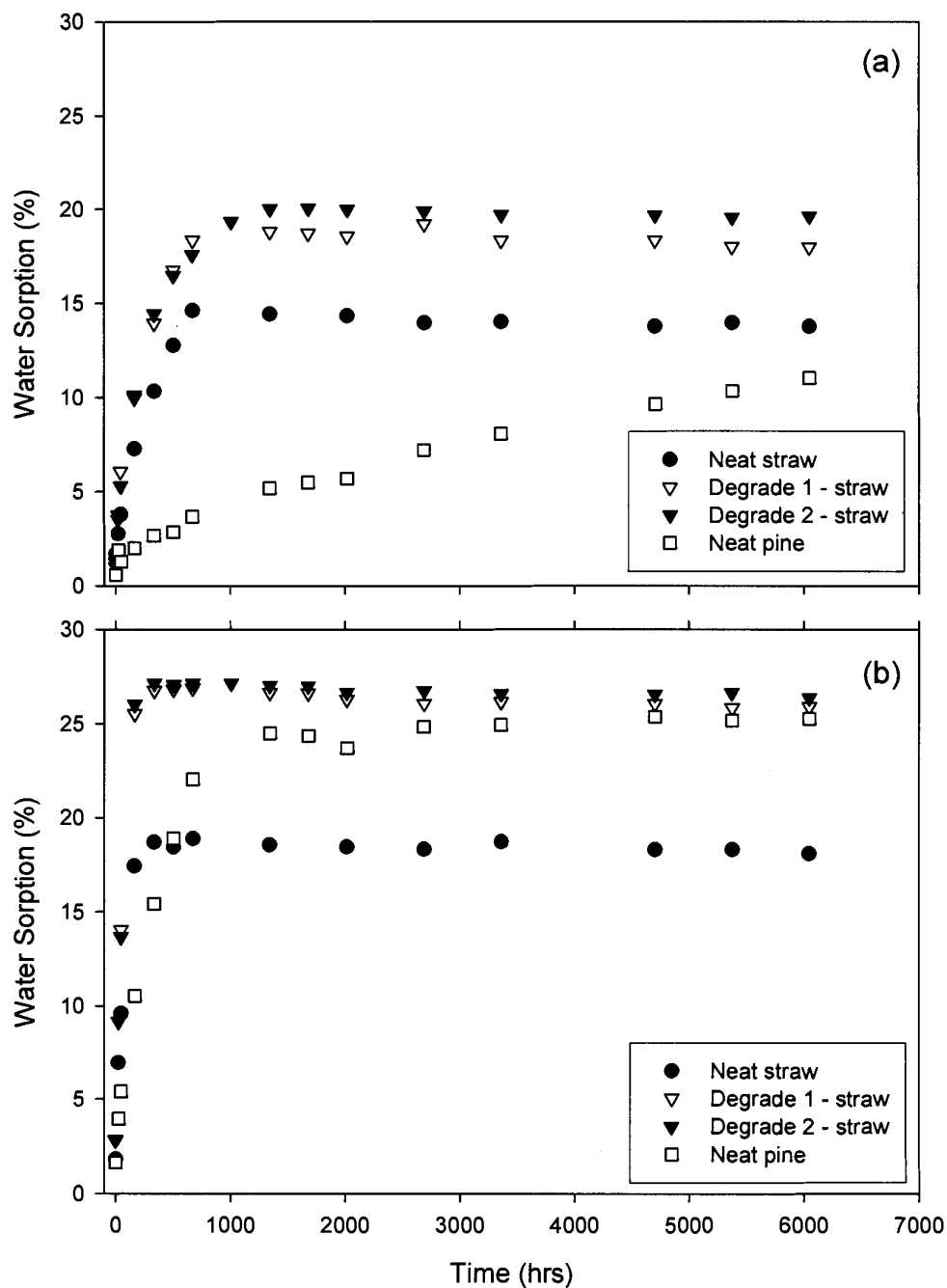


Figure 30. Water sorption of extruded composites: (a) formulation with 55% straw or wood, 38% HDPE, 4% MAPE and 3% OP100; (b) formulation with 75% straw or wood, 18% HDPE, 4% MAPE and 3% OP100.



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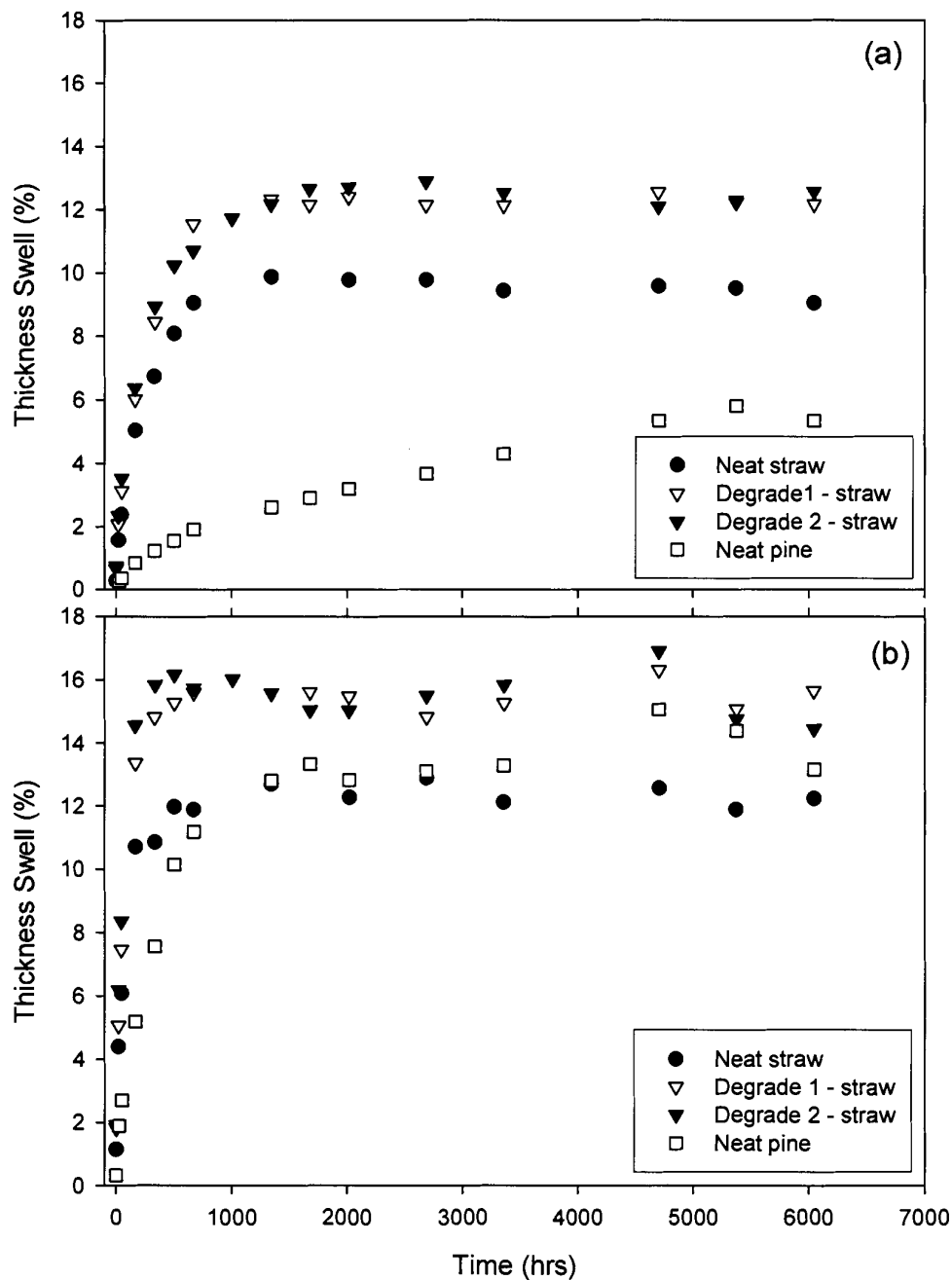


Figure 31. Thickness swell of extruded composites: (a) formulation with 55% straw or wood, 38% HDPE, 4% MAPE and 3% OP100; (b) formulation with 75% straw or wood, 18% HDPE, 4% MAPE and 3% P100.



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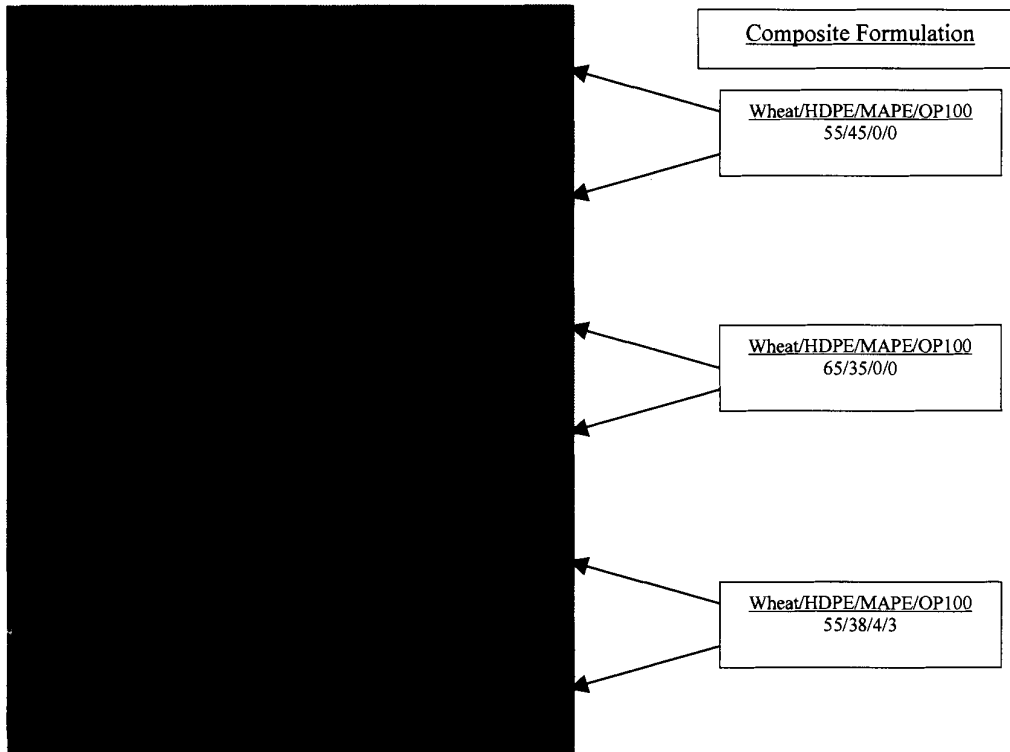


Figure 32. Photograph of Degradate 2-extrudate, based on different formulations. Best results were obtained when 65% (by weight) wheat straw was incorporated in the formulation (see two samples in center). Two samples at bottom show undesirable "snake skin" appearance of extrudate.

In the composites with lower fiber filler content, water sorption of the composite incorporating neat straw was higher than that of a wood-plastic composite (Figure 30a). The reason may be that straw contains more cellulose and less hydrophobic lignin than wood (Sundstøl and Owen 1984, Fengel and Wegener 1989). At higher filler levels, water sorption and thickness swell were similar for straw- and wood-based composites, with the neat straw-based composites displaying the lowest water sorption and thickness swell values (Figure 30b, 31b). In general, with the addition of MAPE into a formulation, water sorption may be reduced.

Results for particle-size distribution of neat and degraded straw are presented in Figure 33. The 12-week degraded

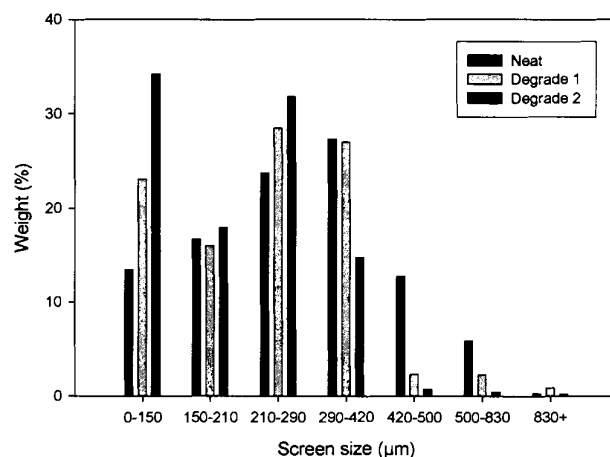


Figure 33. Particle size distribution of neat and degraded straw used in small-scale pilot trial.



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straw (Degrade 2) is primarily composed of smaller particles than the 6-week degraded straw (Degrade 1) which indicates that fungal degradation of the Degrade 2-material was more advanced. This likely had an influence on the extrusion process and mechanical and physical properties of the composite. Maiti and Singh (1986) reported an improvement in yield strength of extruded wood-HDPE specimens with decreasing filler particle-size which was attributed to improved adhesion between filler and thermoplastic since no coupling or dispersing agents were used.

Straw-Plastic Composites based on Straw from Large-Scale Pilot Trial

Inoculated straw for the large-scale trial was provided by the INEEL in sixteen 208.5 L-drums. It was decided to run the large-scale pilot trial in an incubation chamber and not as a field trial (windrows), as suggested in the project proposal. The reason for this diversion from the original proposal is that we considered it highly important (based on the findings from the laboratory studies performed by INEEL) to be able to control the environmental conditions to some degree so that appropriate conditions for fungal growth could be achieved.

An incubation chamber (6.1 x 5.2 x 2.3 (height) m³) was prepared inside an existing plywood structure, which included two small window-like openings on either side of the chamber and an entrance. The inside of the chamber was completely covered with a 2 mm polyethylene foil to prevent contamination of the underlying wood structure. The two small openings and the entrance were also sealed with plastic foil to keep relative humidity at a high level. However, during the course of the incubation, it was found that the temperature increased dramatically, so the plastic foil over the two openings and the entrance was cut open to allow air to circulate more efficiently. A layer of untreated straw was added to the floor of the chamber. Inoculated straw from the supplied sixteen drums was then loosely spread on top of the untreated straw. This procedure was repeated twice, resulting in a total straw pile height of approximately 1 m (uncompressed). The incubation chamber was equipped with a humidifier but without temperature control. It was expected that the temperature inside the chamber would be sufficiently high to enable fungal growth in the wheat straw. Relative humidity and temperature inside the chamber were continuously monitored using a HOBO H8 Pro Series data logger (Onset Computer Corporation, Bourne, MA). Data were downloaded onto a computer using BoxCar Software (Onset Computer Corporation, Version 3.7, Bourne, MA). It was determined that relative humidity in the chamber was on average above 60% with strong fluctuations during incubation (data not shown). Air temperature in the chamber was also subject to strong fluctuations with a minimum temperature of 18°C and a maximum temperature of 32°C, including one cold temperature period of several days in which the nightly temperature in the chamber dropped to 13°C.

The temperature inside the straw pile was also monitored in various places using an immersion thermometer. The pile temperature did not exceed 28°C. The straw pile was turned over on a monthly basis to provide aeration. Additional water was sprayed onto the pile during this process. During the last six weeks of incubation, additional water was added directly to the straw on a weekly basis due to the increased temperature in the chamber. Following three months of incubation, the straw was transferred to the Wood Materials and Engineering Laboratory at Washington State University for processing (e.g., drying and hammer-milling), composite extrusion, and evaluation of composite material properties.

Two formulations, incorporating neat and degraded straw, were extruded into a hollow residential deckboard profile using a commercial extruder at the Strandex® research facility in Madison, WI (Table 19). Formulation #1 was chosen based upon results for mechanical and



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physical properties as well as overall appearance of the extrudate from the small-scale trial. Formulation #2 is similar to standard commercial formulations used in residential deckboard extrusion. For comparison, both formulations were also run with 60-mesh Southern yellow-pine-flour as filler.

Due to poor extrusion performance (i.e., decomposition of the straw in the extruder die, subsequent generation of gases and excessive voids in the SPC), tests to determine the mechanical and physical properties of the straw-plastic composites generated with straw obtained from the large-scale pilot trial were omitted.

The extrusion runs performed at the Strandex® research facility did not proceed satisfactorily due to problems arising from straw degradation as a result of long-term (six months) straw storage between the end of the large-scale incubation and the beginning of the extrusion runs. Therefore, formulations #1 and #2 were run again at the Wood Materials and Engineering Laboratory, however, with the same outcome (Figure 34). In order to address problems with thermal stability of the straw, TGA of the stored straw was conducted (Figure 35). It appears that degradation of the neat, stored straw had occurred which is reflected in the decreased thermal stability of this material between about 200 and 300°C (compare to Figure 27).

The envisioned application of *P. ostreatus* in a farm or outdoor environment represents another practical challenge. In the laboratory-scale research presented here, *P. ostreatus* was applied as liquid inoculum. This allowed homogeneous distribution of actively growing mycelial fragments on the straw, resulting in rapid domination of the culture by *P. ostreatus* and selective removal of the hemicellulose fraction. A fungal strain that could be supplied as an easily revived freeze-dried powder (mycelia or conidiospores), would be easier to distribute and apply in a farm environment using solids mixing techniques. However, our scale-up experience using the (solid) pre-inoculated straw was unsuccessful, and resulted in nonselective degradation of the straw (which is indicative of poor competition of *P. ostreatus* in the straw). This clearly showed that better solid mixing techniques will need to be developed that result in a more homogeneous distribution of the inoculum into the fresh straw material.



Figure 34: Wheat-straw plastic composite made from neat, stored straw showing some defects (right) and standard wood-plastic composite (left). Defects in composite on the right were attributed to straw decomposition in the die during extrusion and subsequent generation of gases.

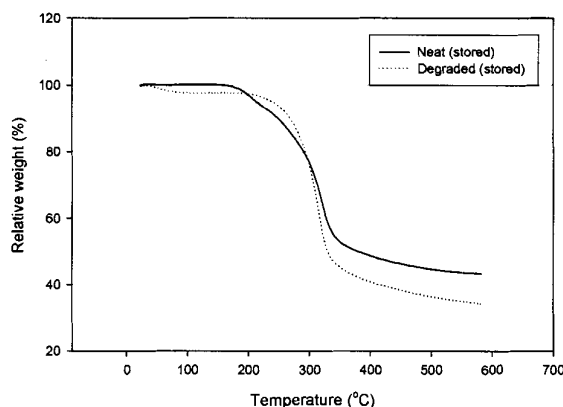


Figure 35. Thermal behavior of neat and degraded straw after six months of storage.



Conclusions

Physical Fractionation of Straw for Gasification/Combustion

Mechanical threshing and separation processes achieved about a 75% pure cereal straw stem fraction, and reduced the cereal straw stem fraction total ash content by 23% and the silica component of the ash by 44%. However, while mechanical separation reduced total straw ash, it was less effective at reducing the alkali content of the ash. Field washing the straw by irrigation had the greatest effect in reducing straw ash alkali content. As little as 13 mm of water applied to straw windrows resulted in a 54% reduction in straw ash potassium and a 55% reduction in straw ash sodium, which are well-known low-temperature eutectic alkali metals. The overall effect of field washing was a 0.94 kg•Mbtu⁻¹ alkali reduction that increased the overall fusion temperature of the ash more than 93°C in an oxidizing atmosphere and more than 149°C in a reducing atmosphere. Grinding the straw to less than 13 mm allowed pneumatic conveyance of the material into the conversion systems and achieved packing densities of greater than 160 kg/m³ for transportation.

Based on the results of this study, any of the processed wheat straw samples could successfully be burned in a fluidized bed in either a combustion or staged combustion process. Washing and separation pretreatments were effective in removing substantial amounts of alkali metals, chlorine, sulfur, calcium oxide, and silica, which improved the combustion process. More importantly, the type or combination of pretreatments applied can selectively and predictably alter biomass constituents to benefit an intended bioenergy conversion end use. From a practical perspective, field washing or selective harvest of straw fractions can be done with existing farm machinery and could fit current farm enterprise practices.

Fungal Pretreatment of Straw in Engineered Storage Systems for Thermoplastic Composites

Laboratory culturing studies showed that by limiting nitrogen and providing sufficient inoculum, it was possible to operate a selective fungal degradation system without prior sterilization of the wheat straw. Using these conditions, unsterile wheat straw stems, with *Pleurotus ostreatus* cultured on straw at constant temperature, produced selective degradation rates and conversions (i.e., depolymerized lignin, consumed hemicellulose, and minimized cellulose removal) that increased with both moisture and inoculum. A regression analysis indicated that system performance was quite stable with respect to inoculum and moisture content after 6 weeks of treatment in laboratory pretreatment culture systems.

Scale-up of fungal pretreatment to a representative engineered storage and pretreatment system presented many challenges with respect to establishing dominant selectively degrading *P. ostreatus* fungal cultures. At the laboratory-scale, the straw *P. ostreatus* inoculum source and inoculation methods as well as straw sterilization both proved effective at establishing stable *P. ostreatus* cultures that dominated indigenous species. However, in the less controlled environment of the larger pretreatment piles, truly dominant *P. ostreatus* cultures could not be fully established using the methods employed. Therefore, larger-scale straw degradation tests did not produce the level of selective fungal degradation that was demonstrated in the laboratory.

The mechanical properties of straw-plastic composites produced with untreated straw were comparable to those of wood-plastic composites. Straw-plastic composites incorporating straw degraded with non-dominant *P. ostreatus* cultures did not have a statistically significant (α -value



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of 0.05) influence on either modulus of rupture or modulus of elasticity of a SPC. However, the mechanical properties of straw-plastic composites produced with untreated straw were comparable to those of wood-plastic composites based on pine flour. In straw-plastic composites with 55% untreated straw, water sorption and thickness swell were higher than that of wood-plastic composites based on pine filler. However, those properties were improved in the straw-based composite when 75% untreated straw was used. Water sorption and thickness swell of straw-plastic composites were inferior to wood-plastic composites at filler levels below 55%. Straw-plastic composites using non-dominant fungal degraded wheat straw demonstrated less resistance to water sorption and thickness swell than straw-plastic composites using untreated straw. This may primarily reflect the fungal degradation of hydrophobic cell wall components (lignin and hemicelluloses) in treated straw, resulting in a relatively more hydrophilic substrate compared to untreated straw. Between 100 and 300°C, fungal degraded straw appeared thermally less stable than non-inoculated straw, but this did not have any apparent effect on the extrusion process. The results obtained in the present study indicate that wheat straw is a promising alternative to wood fillers in the production of extruded thermoplastic composites, pending an improvement in the hydrophobicity of straw-plastic composite at commercially relevant filler levels. As an alternative to wood fillers, untreated straw produced a superior straw composite plastic than straw degraded with non-dominant *P. ostreatus* fungal cultures.

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Appendix A. Project Publications

Hess, J. R., R. L. Hoskinson, T. D. Foust, R. L. Kenney, D. N. Thompson, P. G. Shaw and J. Steciak. (2002). "Multi-Component Harvesting for Whole Crop Utilization," Bioenergy 2002 – Bioenergy for the Environment, September 22-26, 2002, Boise, Idaho.

Hess, J. R., R. L. Hoskinson, D. N. Thompson, J. Steciak, D. Krapas, and B. Cosby. (2004). "Selective harvest and pretreatment of cereal straw residue for bioenergy conversion." Proceedings of the 2nd World Conference and Technology Exhibition on Biomass for Energy and Industry, Rome, Italy, May 10-14, Paper OC10.3.

Hoskinson, R. L., D. N. Thompson, T. D. Foust, D. R. Grant, J. R. Hess, T. P. Houghton, J. A. Lacey, and P. G. Shaw. (2002). "Distributed Fungal Harvest of Higher Value Wheat Straw Components." Meeting Paper No. 02-6013. ASAE Annual International Meeting, Chicago, Illinois. July 28-31, 2002.

Hoskinson, R. L., and J. R. Hess. (2004). "A Single Pass Multi-component Harvester for Small Grains." Meeting Paper No. 04-1161. ASAE Annual International Meeting, Ottawa, Canada. August 1-4, 2004.

Houghton, T. P., D. N. Thompson, J. R. Hess, J. A. Lacey, M. P. Wolcott, A. Schirp, K. Englund, D. Dostal, F. Loge. (2003). "Fungal Upgrading of Wheat Straw for Straw-Thermoplastics Production," 25th Symposium on Biotechnology for Fuels and Chemicals, May 4-7, 2003, Breckenridge, CO.

Houghton, T. P., D. N. Thompson, J. R. Hess, J. A. Lacey, M. P. Wolcott, A. Schirp, K. Englund, D. Dostal, F. Loge. (2004). "Fungal Upgrading of Wheat Straw for Straw-Thermoplastics Production." Applied Biochemistry and Biotechnology 113-116: 71-93.



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Schirp, A., F. Loge, K. Englund, M. P. Wolcott, J. R. Hess, T. P. Houghton, J. A. Lacey, D. Thompson. (2004). Pilot-scale production and material properties of extruded straw-plastic composites. Manuscript in preparation for submission to Holz als Roh- und Werkstoff.

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Hoskinson, R. L., J. R. Hess, K. L. Kenney, J. M. Svoboda, T. D. Foust, D. N. Thompson, and W. T. McKean. (2004). "Method and Apparatus for Selectively Harvesting Multiple Components of a Plant Material." Patent No. US 6,729,951 B2. May 4, 2004.

Appendix B – Gasification/Combustion Test Facility

Fluidized Bed Research and Development Facility

Energy Products of Idaho, Inc. (EPI) owns and operates a 3.0 MBtu/hour (nominal HHV Output) pilot-scale atmospheric, bubbling type fluidized bed combustion system. The facility is dedicated to determining optimum system operating conditions for each client's process needs through the gathering of environmental and engineering design information.

The fluid bed pilot unit contains nine square feet of bed area and twenty-six feet of freeboard above the nozzles. Seven sets of overfire air (OFA) ports penetrate the refractory of the freeboard space, with each set housing eight individual ports. Each set of OFA ports is individually controlled and may be used to inject atmospheric air, Flue Gas Recirculation (FGR), and/or reagents for SNCR NO_x abatement. For this particular study, a refractory choke was installed to reduce the bed size to a 1 ft-4 in. x 1 ft-4 in. area. This "choked," or reduced, bed allowed for gasification modeling of the fuel within the bed and staged combustion in the vapor space.

A horizontal refractory-lined duct, approximately three feet long, connects a refractory cyclone to the combustion system. Flue gases may exit the cyclone through a short outlet stack, or the flow may be isokinetically pulled through a heat exchanger and baghouse via an ID fan for stack gas emissions monitoring and baghouse ash sampling. Part of this flue gas flow or the entire amount may be directed to the FD fan as combustion air and used as "Flue Gas Recirculation" (FGR). FGR from the outlet of the baghouse may also be directed to the OFA system to each individual level of OFA.

The fuel feed system consists of a variable speed, live-bottom metering bin with a rake-back conveyor that delivers a non-compressed layer of fuel to a take-away conveyor. The take-away conveyor distributes the fuel onto a flex-o-wall conveyor for discharge into an over-bed feed tube. A vibratory metering system feeds limestone or other additives on the flex-o-wall conveyor with the fuel. A pneumatic conveyance system, using high-pressure air from the FD Fan, may also be used for injection of finer materials directly into the fluid bed. Fuel is delivered to the air induction system with a fixed speed rotary feeder prior to the flex-o-wall conveyor. For this study, the ground wheat straw was fed directly into the fluidized bed region with the pneumatic conveyance system. The pelletized wheat straw was fed into the combustor via the above-bed gravity feed system. Limestone was also fed to the combustor with the above-bed gravity feed system.

A high pressure FD fan capable of 150 in. of static water pressure delivers air to the fluidizing nozzles, overfire air ports and pneumatic fuel feed system. A propane-fired preheat burner tees into the under fire air plenum and provides 800°F air through the fluidization nozzles. A propane-fired above-bed burner is also utilized to preheat the vapor space of the combustion system. In approximately three hours the bed is heated to more than 800°F, at which time solid fuel can be added to bring the combustion system up to operating temperature. On shutdown at the end of the test day, the combustion system remains hot enough to be fired the following test day with just the addition of in-bed propane or solid fuel. Propane or any variety of fuel oils may be metered in-bed or in the vapor space as auxiliary fuel for the staged combustion of low quality solid fuels.

Noncombustible tramp material passes through the active bed region and remains in the inactive bed until cycled out through a timed slide gate at the bottom of the vessel. The sand





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and tramp material are conveyed through a water-cooled auger to a screen and magnetic separator for removal. Clean sand is metered back into the vessel on a continuous basis, with the combustion system in full operation.

The entire system is fully instrumented to provide all pertinent combustion and emissions data. System pressures, pressure drops, flow rates, temperatures, and emission concentrations are continuously monitored and recorded throughout testing.

Constant Monitoring Equipment

The following EPA certified analyzers are maintained by EPI for continuously monitoring stack emissions from the pilot plant.

<u>Gas</u>	<u>Analyzer</u>	<u>Detection Method</u>
SO ₂	Western Research Model 721A	Ultraviolet
NO/NO _x	Thermo Electron Corporation Model 10A	Chemiluminescence
O ₂	Taylor Servomex 0A580	Paramagnetic
CO	Horiba Pir 2000	Nondispersive Infrared

Flue gas samples may be secured at the inlet or outlet of the baghouse. The analyzers are located in a control room and spanned with Protocol One gases, as necessary, throughout the tests. A sample conditioning tray removes water from the flue gas prior to introduction to the analyzers via a Teflon line. The analyzers serve to monitor the stability of the process and to provide emissions performance data.

Feedstock Testing of Treated Straw – Combustion and Gasification Test Photographs

Combustion and staged combustion (gasification in-bed/staged combustion above-bed) and emissions characteristics of untreated, washed and separated, washed and unseparated, and unwashed and separated straw were performed at Energy Products of Idaho in Coeur d'Alene, Idaho. The following figures (B-1–B-23) provide a photographic record of these tests.



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Figure B-1. Exterior view of the EPI pilot-scale fluidized bed facility in Coeur d'Alene, Idaho.



Figure B-2. Preparing to dump fuel from a supersack, hauled from covered storage, into the metering bin.



Figure B-3. Metering bin covers removed to check the pulverized straw feed. Note the dust. The pulverized straw did not clog the fuel feed system. This light fuel was injected directly into the bed during combustion tests.

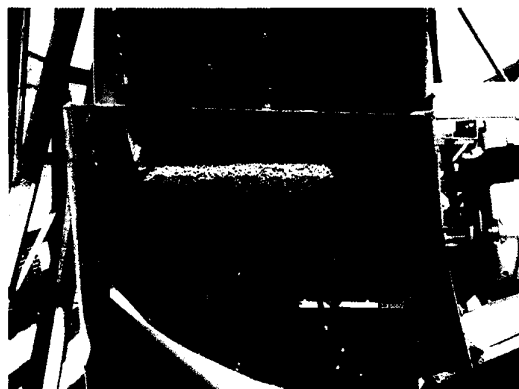


Figure B-4. Metering bin covers removed to see the slow cascade of pelletized straw into the fuel feed conveyor. Note the absence of dust compared with the pulverized straw. The heavier pelletized fuel was injected above the fluidized bed during combustion tests.



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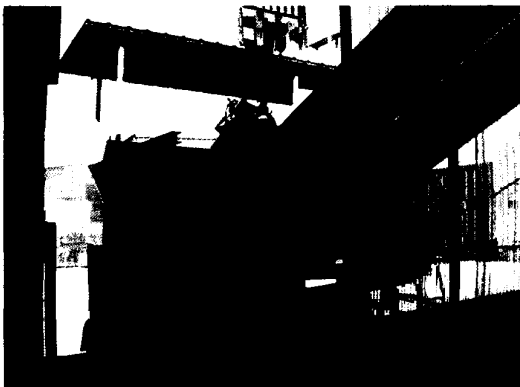


Figure B-5. Interior view of the fuel feed conveyor.



Figure B-6. Interior view of the pilot-scale fluidized bed facility. In-bed fuel feed is delivered through the pipe that enters the fire box on the platform between the yellow railings.



Figure B-7. Shoveling dolomite, a bed additive that raises the slagging temperature of straw ash, into a metering bin. One goal of the combustion task is to minimize the amount of dolomite that is needed to prevent bed agglomeration.



Figure B-8. Bed media is sifted to remove small agglomerations and foreign objects, and recycled. Bed change-out occurs periodically during testing. This is a view of the auger that delivers bed media to the sifter.

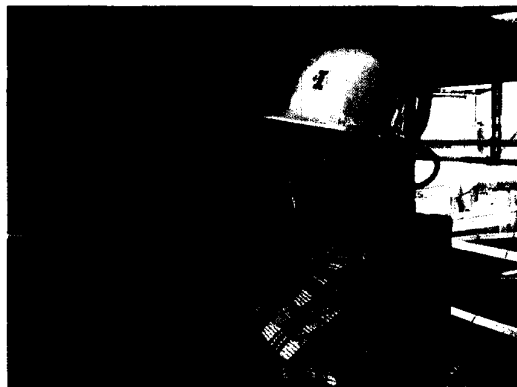


Figure B-9. At the in-bed observation port.

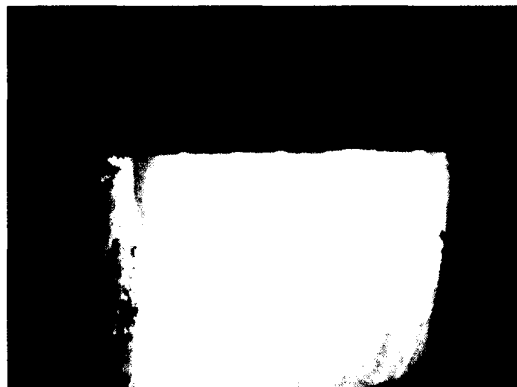


Figure B-10. In-bed view of propane burning to warm up the bed. Note the uniform flames.



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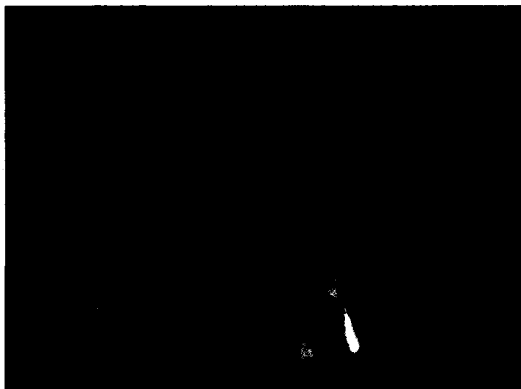


Figure B-11. In-bed view of pulverized straw during combustion. Note the streak lines caused by burning pieces of straw.

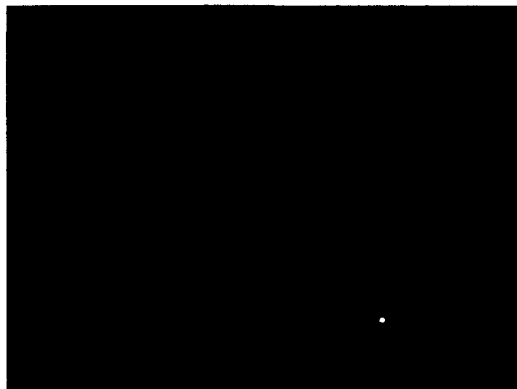


Figure B-13. In-bed view of pelletized straw during combustion. Note the wide streak lines compared with the finer streak lines during pulverized straw combustion.

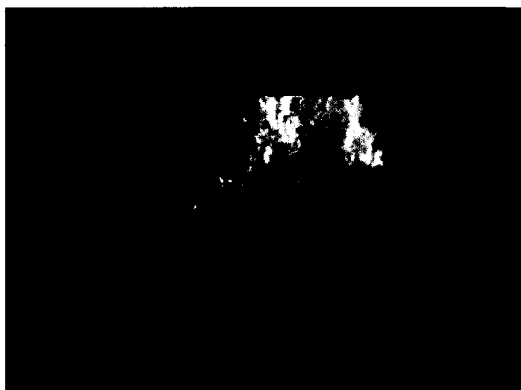


Figure B-12. In-bed view of pulverized straw during staged combustion (gasification in the bed, with above-bed air added to burn the producer gas). Note the increase in streak lines caused by burning fuel particles. Gasification conditions are achieved by increasing the fuel feed rate while maintaining the same air flow rate used for combustion conditions.

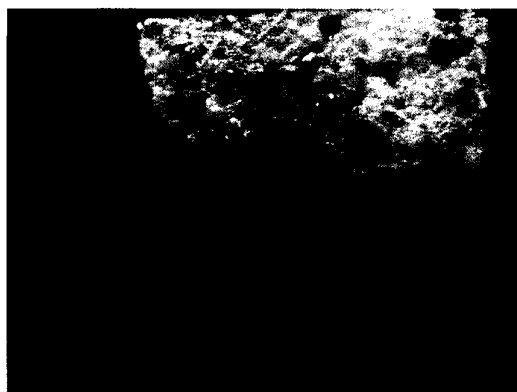


Figure B-14. In-bed view of pelletized straw during staged combustion when bed agglomeration occurred because of an accidental fuel overfeed. Note the absence of streak lines, which means that the bed is no longer fluidized. Both the sandy bed media and fuel pellets are visible.



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Figure B-15. Above-bed view of pulverized straw combustion.



Figure B-16. Above-bed view of a sheathed thermocouple during pulverized straw combustion tests. The feathery deposits are soft fly ash, not slag. Fly ash continually deposited on the sheath, and eroded away, during the testing. Note few streak lines; most of the fuel was burned in the bed as planned. The stationary specks are backlit dust particles on the viewport glass.

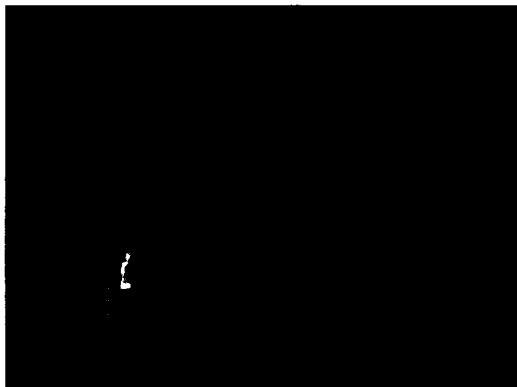


Figure B-17. Above-bed view of staged combustion tests with pulverized straw. Note the many streak lines. To achieve gasification conditions with pulverized straw, the fuel was fed both in-bed and above-bed.

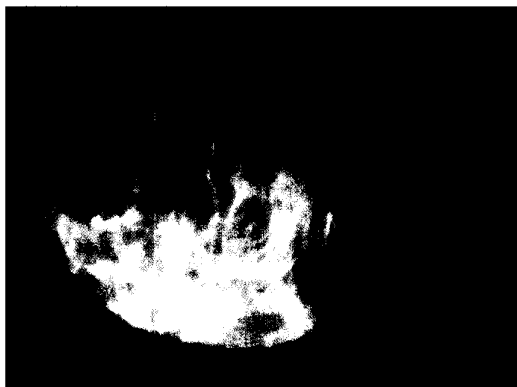


Figure B-18. Above-bed view of pelletized straw during combustion tests. The uniform flame with no streak lines indicates that the pellets are consumed in the bed, as planned. Feathery fly ash deposits on the thermocouple sheath and along the viewport tunnel formed and eroded continually.



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Figure B-19. Double-checking pressure and temperature readings during steady-state operation.



Figure B-22. Double-checking the stack pressure reading during steady-state operation.



Figure B-20. Control room operations include logging instrument readings for pressure, temperature, and CEMS, and measuring the fuel moisture content.



Figure B-21. Preparing the ice bath for gas conditioning. The ice bath condenses water from the exhaust gases before CO and NO_x concentrations are measured.

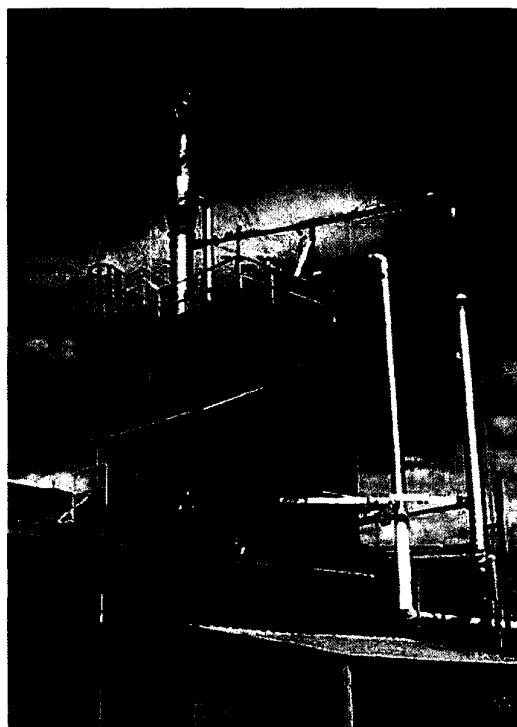


Figure B-23. Note the plume of steam exiting the stack.